RETURN PROMPTLY TO
RESEARCH LIBRARY
PARKE, DAVIS & CO.
ANN ARBOR

HENRY FORD HOSPITAL MEDICAL BULLETIN

Vol. 9 March, 1961 No. 1

INTRA-ARTERIAL CHEMOTHERAPY OF MALIGNANT DISEASES*

R. W. Talley, M.D., V. K. Vaitkevicius, M.D.,
M. J. Brennan, M.D., and J. E. Kelly, M.D.**

During the past two decades the interest in the application of chemicals to the patient with inoperable, radiation-resistant, malignant disease has markedly increased. Many compounds have been evaluated and those which have shown efficacy have been limited in usefulness by their general systemic toxicity. Even though the quest for new, relatively non-toxic chemicals is progressing at a rapid rate, there is no evidence of which we are aware that such an agent is out of the test tube.

However, by the administration of these agents intra-arterially their systemic toxicity may be decreased. Several methods have been employed and, for sake of simplicity, we have divided these into the following three categories: (1) acute infusion, (2) acute perfusion, and (3) chronic infusion with antidote. The technique of acute infusion was first described by Klopp[1] and results will not be discussed here but in a subsequent publication.

RESULTS

The acute perfusion technique as first described by Creech and co-workers[2] has been most applicable in tumors localized in an extremity in which amputation or other surgical technique could not be employed. However, perfusion of the pelvis, lungs, brain, and other vascular isolatable organs are under evaluation. In a recent Perfusion Conference held at Tulane University in New Orleans, the results of many investigators were presented and, for a comprehensive survey, the reader is referred to the publication[3] of this conference.

In perfusion of an extremity the vascular supply of the area is isolated surgically with cannulization of the major artery and vein and tourniquet applied proximal to these. The chemotherapeutic agent, usually an alkylating agent, is administered by a sigmamotor pump extracorporeal system with bubble oxygenator. The drug employed in all of our cases except two has been nitrogen mustard at a dose of 0.6 to 1.0 mg./kg. We have found that, by careful monitoring of the perfusion system and the patient's blood from a distant sight with chromate labeled red cells, as well as keeping the perfusion system pressure (Austen and co-workers)[5] below the patient's average blood pressure, systemic toxicity may be kept to a minimum.

The seven patients undergoing such procedure are listed in Table I. Two of these patients (#3 and 7) can be classified as prophylactic perfusions; the rationale for such prophylactic perfusion has been convincingly put forth by Stehlin.[6] Patients #2 and #5 had worthwhile responses. Patient #5 is a 31-year-old, white female with recurrent melanoma (Fig. 1) in right epitrochlear node and right axillary nodes. Axillary node dissection followed by perfusion with nitrogen mustard at 1.0 mg./kg. resulted in 7 cm. decrease in circumference of arm. There has been no evidence of regrowth of the tumor five months after the perfusion.

---

*Presented at Regional Meeting, American College of Physicians, Flint, Mich., December 3, 1960.
**Department of Medicine, Division of Oncology.

TABLE I

PERFUSION RESULTS WITH ALKYLATING AGENTS

Patient	Diagnosis	Perfusion Site	Drug and Dose	Toxicity	Other RX	Results
1. M.L.	Ca. Bladder	Pelvis	TESPA 60 mg.	WBC ↓ 500	—	Subj., 4 wk
2. R.W.	Ewing's Sarcoma	Left leg	HN_2, 0.6 mg./kg.	WBC 2,000	Radiation 6,000 r	No recur in 9 months
3. K.S.	Melanoma	Left leg	HN_2, 0.8 mg./kg.	N & V	Radical groin dissection	No recur in 9 months
4. H.Z.	Squamous cell ca.	Right leg	TESPA 60 mg.	—	Radical groin dissection	No effect
5. M.L.	Melanoma	Right arm	HN_2, 1.0 mg./kg.	N & V	Axillary dissection	Obj. rem. 5 months
6. H.P.	Melanoma	Right leg	HN_2, 0.8 mg./kg.	Sciatic nerve paresthesias	Inguinal dissection	No effect
7. M.S.	Melanoma	Right leg	HN_2, 0.8 mg./kg.	Vesicular dermatitis, Peroneal nerve palsy	Inguinal dissection	Prophylac. only — no recur in 5 months

9-13-60 10-5-60

Figure 1

Patient #5, M. L., showing site of epitrochlear metastases of a recurrent melanoma. The circumference of upper arm on September 13, 1960, was 35 cm. and on October 5, 1960, it measured 27 cm.

Patient #2 is a 16-year-old boy with Ewing's sarcoma of the left femur (Fig. 2). Local acute perfusion with nitrogen mustard at 0.6 mg./kg. resulted in decrease in pain and swelling of leg. This was followed by 6,000 r delivered by a cobalt teletherapy unit. There has been no recurrence of disease eleven months later. It is impossible to say whether such combined therapy has been or will be any more efficacious than radiation therapy alone, but it is planned to treat additional patients in such a manner if the opportunity arises.

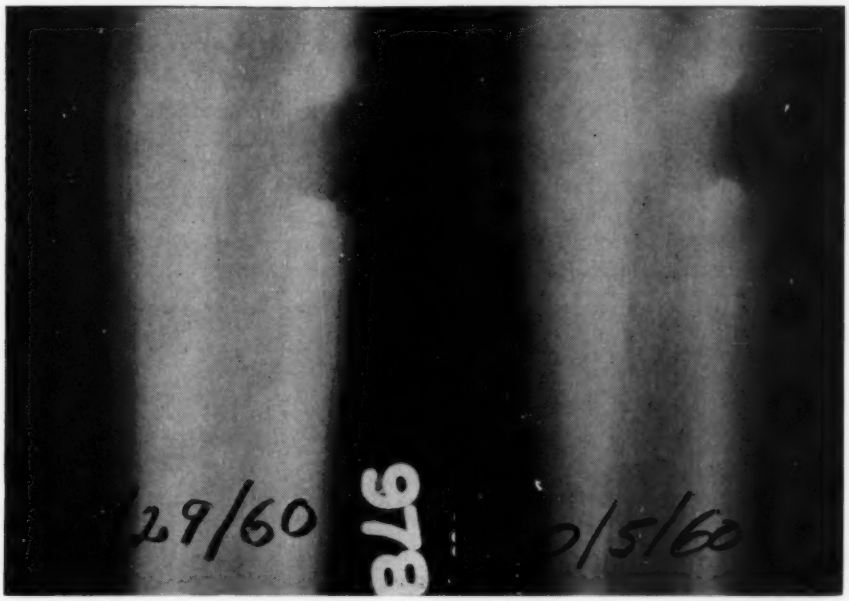


Figure 2

Roentgenograms of left femur in patient #2, R. W., reveal typical "onion peel" appearance of left femur. Large defect is the biopsy site. There has been complete disappearance of the "onion skin" appearance in the film of 10/5/60 and, expect for thickening of cortex, the bone appears normal. This film was taken 8 months after nitrogen mustard perfusion and radiation therapy.

The third technique being discussed, chronic arterial infusion of antimetabolite with systemic metabolite therapy and the rationale for such, was recently described by Sullivan.[4] In this technique, an antimetabolite such as amethopterin is administered via a constant arterial infusion to a localized area by a Barron pump.[7] Simultaneously, the patient is given relatively large doses of the normal metabolite such as folinic acid to prevent or delay systemic toxicity. Amethopterin is administered in a dose of 50 mg./day via arterial infusion for at least four days and folinic acid is given in intermittent daily doses of 9-36 mg. intramuscularly. Fifteen patients have been treated by this technique and are presented in Tables II, III, and IV according to the area infused.

TABLE II

ARTERIAL INFUSION WITH AMETHOPTERIN

A. Pelvic Infusion via Hypogastric Arteries

Patient	Diagnosis	Total Dose (Milligrams)	Response Subj.	Response Obj.	Minimal WBC	Other Toxicity
1. G.S.	Ca. Cervix	310 mg.	††† 5 mo.	† 2 mo.	950	Diarrhea, stomatitis
2. M.K.	Ca. Cervix	200 mg.	† 1 mo.	†† 1 mo.	700	Staph-entero colitis
3. R.C. (a)	Ca. Colon	175 mg.	0	†	5450	Diarrhea
(b)	Ca. Colon	125 mg.	0	0	2000	Diarrhea

TABLE III

ARTERIAL INFUSION WITH AMETHOPTERIN

B. Via Internal Carotid

Patient	Diagnosis	Total Dose (Milligrams)	Response Subj.	Response Obj.	Minimal WBC	Other Toxicity
4. H.R.	Glioblastoma	350 mg.	0	†	3500	—
5. R.M.	Glioblastoma	525 mg.	† 1 mo.	†† 1 mo.	1450	Diarrhea
6. P.D. (a)	Meningiosarc.	100 mg.	—	—	6000	—
(b)	Meningiosarc.	250 mg.	—	—	750	Dead on 7th post-inf. day
7. H.G.	Astrocytoma	250 mg.	—	—	8200	Death 1 day post-infusion

TABLE IV

ARTERIAL INFUSION WITH AMETHOPTERIN

C. Via External Carotid

Patient	Diagnosis	Total Dose (Milligrams)	Response Subj.	Response Obj.	Minimal WBC	Other Toxicity
8. D.R. (a)	Ca. Floor of Mouth	490 mg.	†††† 1 mo.	††† 1 mo.	4200	Stomatitis
(b)	Ca. Floor of Mouth	412.5 mg.	††	†††	1450	Stomatitis
9. H.H.	Ca. Tonsil	350 mg.	—	—	50	Death- Leukopenia, acute pulm. edema
10. L.A.	Ca. Pharynx	350 mg.	†	—	2600	Stomatitis & severe anx'y
11. R.K.	Ca. Tongue	350 mg.	†	† 3 wks	4800	CVA ?
12. S.M. (a)	Ca. Tongue	335 mg.	†† 3 wks	† 3 wks	3100	Stomatitis,
(b)	Ca. Tongue	290 mg.	†† 7 wks	†† 7 wks	1250	diarrhea, & bronchopneu.
13. J.K. (a)	Osteogenic Sarcoma	300 mg.	††	†	3800	Stomatitis
(b)	Zygoma.	300 mg.	†	†	2800	Stomatitis
(c)		200 mg.	††† 5 wks	†† 4 wks	4350	—
14. J.E.	Melanoma	200 mg.	—	—	4200	Inf. D/C'd at patient req.
15. V.D.	Ca. Breast, scalp metast.	240 mg.	†	†	3300	Stomatitis Hemorrhage

Three hypogastric artery infusions for pelvic carcinoma (Table III), resulted in production of significant subjective and objective improvement in one patient with carcinoma of the cervix, definite but limited benefit in another patient with carcinoma of the cervix, and no help in a patient with carcinoma of the rectum.

Four patients with various brain tumors were infused via the internal carotid artery as shown in Table IV. Only one patient (H.R.) obtained objective benefit with decrease of intracranial pressure to normal and disappearance of pain, but because

of pre-existing brain damage, this response was considered to be of limited value.

Eight patients have been infused via the external carotid artery and objective and subjective benefits have been noted in six, but of short duration in all (Table IV). Sullivan[4] reported significant prolonged regressions in three of eighteen patients treated similarly. Prolonged regressions were not noted in our series, but worthwhile palliation was obtained in four patients. The most gratifying result was obtained in patient #8, a 50-year-old man with recurrent radiation-resistant carcinoma of the floor of the mouth with direct extension through the mandible, in whom the infusion was carried out on two occasions. After both infusions there was dramatic diminution of the tumor mass (Fig. 3), but after the first infusion there was rapid regrowth of the

12-9-59 2-2-60

Figure 3

Patient #8, D. R. (Table III), with carcinoma of floor of the mouth with direct extension through the mandible. Photograph prior to Amethopterin infusion and appearance of lesion 4 days after completion of second infusion.

tumor. Because of the rapid growth of the tumor after the first infusion and the obvious fact that not all of the tumor had responded after the second infusion, a radical excision of the anterior half of the mandible and tongue was performed. The pathologic specimen did not reveal any recognizable viable malignant cells in the marginal lymph nodes and edges of the excised specimen. There has been no recurrence in eleven months and the patient is now undergoing plastic reconstruction of the face. It is impossible to say whether or not such a surgical procedure would have been possible without chemotherapy, however, this patient had been considered inoperable by several examiners.

TOXICITY

Toxicity associated with alkylating agent perfusion has been summarized recently by Reemstra.[8] Gastrointestinal reactions, leukopenia, nerve injuries, skin erythema,

and skin ulceration were the most common complications noted in a series of 231 perfusions. All of these were noted in our small series of seven patients. The most marked leukopenia was noted in patient #1, in whom pelvic perfusion was undertaken, and had an approximate 70% leak as calculated by chromium$_{35}$-tagged red cells. The toxicities are summarized in Table I. Our series is too small to give any true incidence of these complications.

The major toxicity associated with the chronic infusion of amethopterin was that of bone marrow depression with leukopenia and thrombocytopenia. Ulceration of the buccal mucosa in external carotid infusions and of diarrhea in hypogastric infusions were a problem in about one-half of each group. There were two deaths in association with severe leukopenia.

The dose relationship of folinic acid to leukopenia in the amethopterin treated patient is of great importance and leukopenic responses in relation to three dose levels of folinic acid is demonstrated in Fig. 4, 5, and 6. The maximal leukopenia in patients receiving less than 35 milligrams of folinic acid a day occurred by the 8th day, in the majority of patients. Recovery to normal levels in all of these patients was about the same. Patients receiving 36 or more milligrams a day did not drop to as low a level and recovered more rapidly, however, the differences were not great. Two fatal

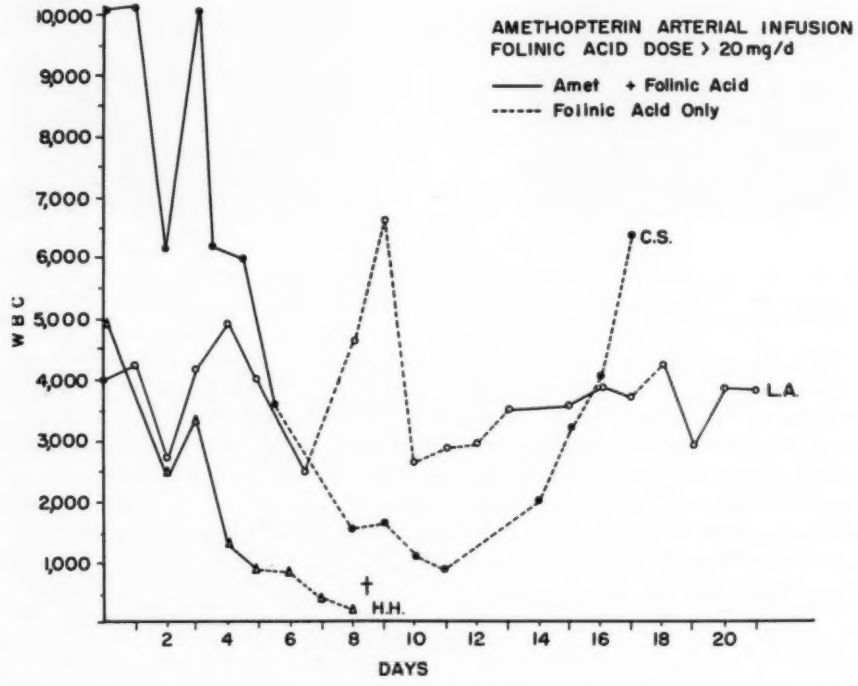


Figure 4

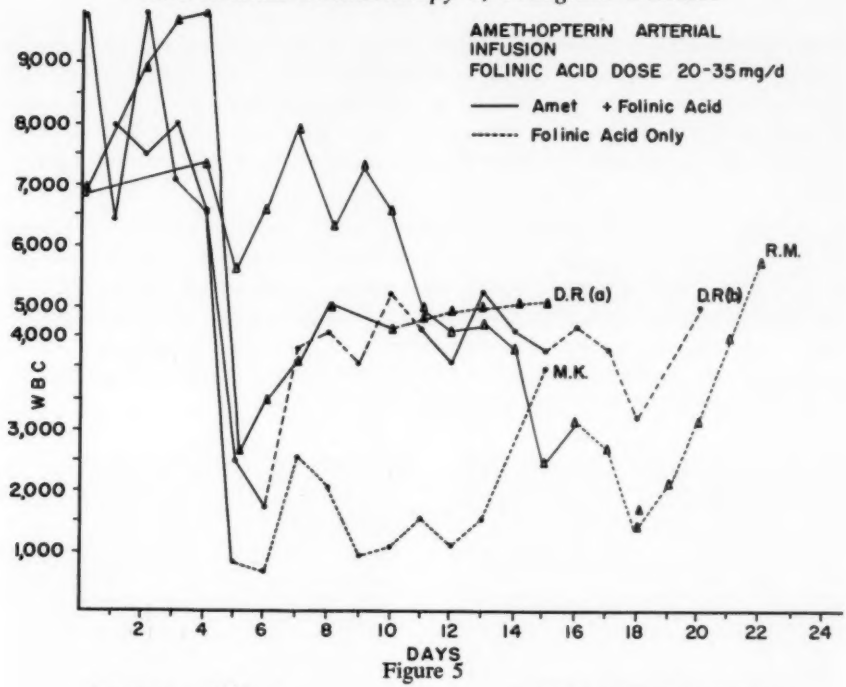


Figure 5

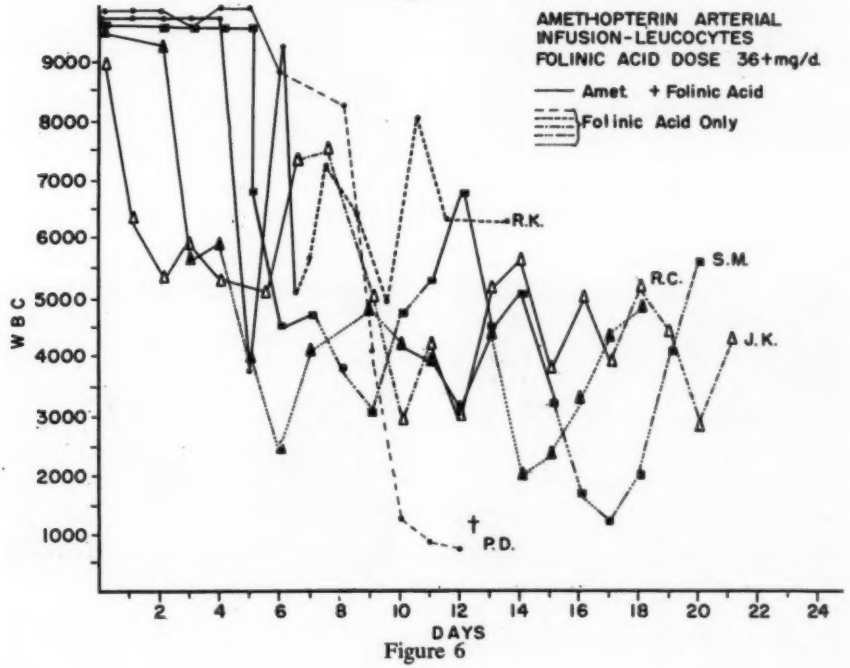


Figure 6

leukopenic reactions occurred and, in one patient, P. D., in whom the amethopterin was discontinued when the peripheral white blood count was still 8,400. Partly because of this, we now rely heavily on cytological changes in the bone marrow which demonstrate progressive evidence of megaloblastic changes in the erythroid series, increase in granulocytic-erythroid ratio, and disappearance of immature granulocytes.

In Fig. 7 (a-h.) we see atypical erythroid and granulocytic elements developing in patient #4 (H.R.), receiving 50 milligrams amethopterin daily via the internal carotid artery and 36 milligrams of folinic acid intramuscularly daily. The first typical megaloblastic cell suggesting a thymine deficiency[9] is seen at 28 hours. Approximately 48 hours is required for disappearance of the major abnormalities. It is suggested from this that a greater dose than 36 milligrams of folinic acid is necessary to prevent hematopoietic changes induced by amethopterin.

Figure 7a - 7b

Series microphotographs of marrow elements from patient H. R.

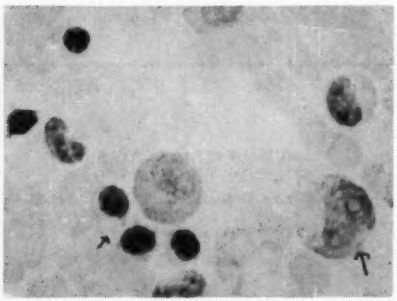


Figure 7a

Control specimen showing normoblasts and myelocytes.

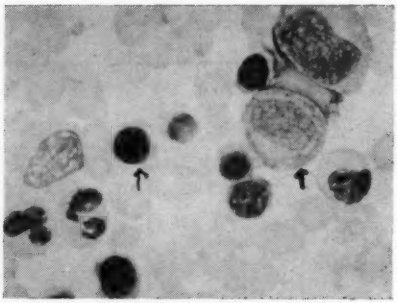


Figure 7b

4-hours — essentially normal normoblasts and myelocytes.

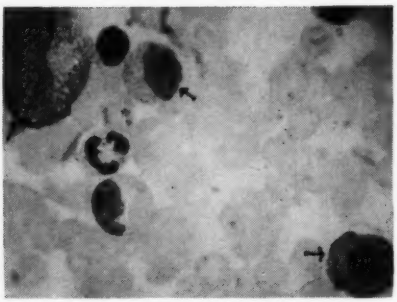


Figure 7c

16 hours — large normoblasts, with loosening of nuclear chromatin, and giant metamyelocytes.

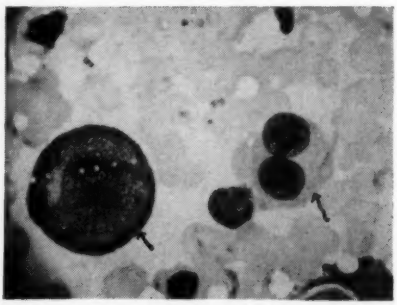


Figure 7d

28 hours — typical megaloblast, double nucleated normoblast.

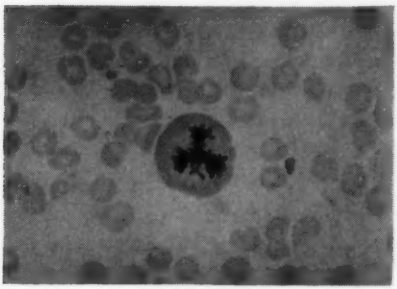


Figure 7e
36 hours — tripolar mitotic figure.

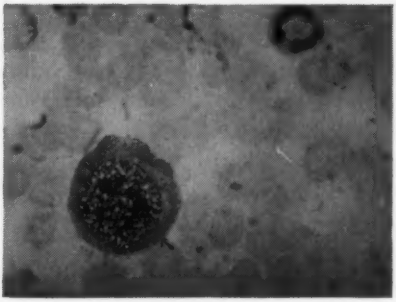


Figure 7f
48 hours — typical megaloblast, sparseness of myelocytes.

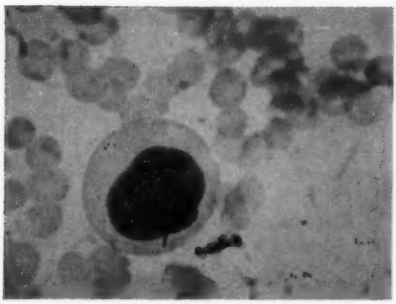


Figure 7g
24 hours after discontinuance of Amethopterin — megaloblast with increasing condensation of nuclear chromatin.

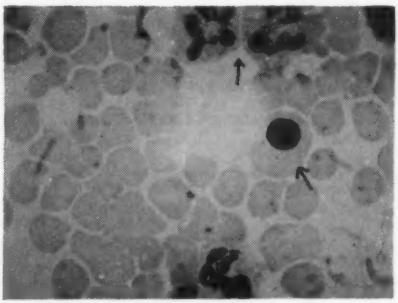


Figure 7h
48 hours after completion — macrocytic normoblast and hypersegmented granulocytes.

SUMMARY

Intra-arterial administration of alkylating agents and antimetabolites have become more practicable with the development of newer techniques and, in occasional instances, have produced effective palliative results. However, the toxicity from any of our currently available chemotherapeutic agents is still a significant hazard even from arterial administration techniques.

Perfusion of an isolated vascular area appears to be beneficial primarily in tumors in an extremity, though development of techniques for perfusion of other areas are in progress. However, this is primarily a surgical problem and we depend on our surgical colleagues for developments in this area.

The administration of amethopterin intra-arterially to 15 patients while simultaneously administering folinic acid has been of definite but limited value in 6 of 12 patients with carcinoma in the head or neck region, and 1 of 3 patients with pelvic carcinoma. No definite benefit was noted in patients with brain tumors. The toxicity

of such therapy is significant. Amethopterin infusion should be undertaken only in patients willing to accept a definite hazard. It must be stressed, however, that in many patients there is no other therapy available. If procedures such as those described here are undertaken with caution, some patients will obtain a degree of palliation not previously available to them.

ACKNOWLEDGMENTS

We wish to acknowledge and thank our surgical colleagues for the assistance and advice in the treatment of these patients, and especially for their skill in placement of catheters in the proper arteries. They are: Dr. E. Szilagyi, Dr. J. G. Whitcomb, Dr. R. F. Smith of Vascular Surgery, Dr. R. S. Knighton, Dr. A. B. Eisenbrey of Neuro-Surgery, and Dr. Paul Hodgkinson of Gynecology.

REFERENCES

1. Klopp, C. T.: Fractionated intra-arterial cancer chemotherapy with methyl bis amine hydrochloride; preliminary report, Ann. Surg. 132:811, 1950.

2. Creech, O., Jr.: Experience with isolation-perfusion techniques in the treatment of cancer, Ann. Surg. 149:627, 1959.

3. Conference on Cancer Chemotherapy by Regional Perfusion, Cancer Chemotherapy Reports, 10, Dec. 1960.

4. Sullivan, R. D., Miller, E., and Sikes, M. P.: Antimetabolite-metabolite combination cancer chemotherapy, Cancer 12:1248, 1959.

5. Austen, W. G., Monaco, A. P., Richards, G. S., Baker, W. H., Shaw, R. S., and Raker, J. W.: Treatment of malignant pelvic tumors by extracorporeal perfusion with chemotherapeutic agents, New England J. New England J. Med. 261:1037, 1959.

6. Stehlin, J. S., Jr., Clark, R. L., White, E. C., Smith, J. L., Jr., Griffin, A. C., Jesse, R. H., Jr., and Healey, J. E., Jr.: Regional chemotherapy for cancer; experiences with 116 perfusions, Ann. Surg. 151:605, 1960.

7. Tucker, J. L., and Talley, R. W.: Prolonged intra-arterial chemotherapy for inoperable cancer; a technique, Cancer (In press).

8. Reemstra, K.: Complications of perfusion, Cancer Chemotherapy Reports, 10, p. 75, Dec. 1960.

9. Vilter, R. W., Harrigan, D., Mueller, J. F., Jarrold, T., Vilter, C. F., Hawkins, V., and Seaman, A.: Studies on the relationships of vitamin B_{12}, folic acid, thymine, uracil, and methyl-group donors in pernicious anemia and related megaloblastic anemias, Blood 5:695, 1950.

HEMORRHAGIC COMPLICATIONS OF POLYCYTHEMIA VERA*

JOSEPH P. ABRAHAM, M.D.,† SHIRLEY A. JOHNSON, Ph.D.,‡
ORHAN N. ULUTIN, M.D.,§ AND M. JUNE CALDWELL, B.A.¶

The predominant interest and therapeutic effort has been directed at the thrombotic complications of polycythemia vera. This is proper inasmuch as throbosis is the chief source of disability in the disease. This tendency to thrombosis is readily explained by the increased blood viscosity, the high platelet count and the slowed rate of blood flow.

Another major source of disability and occasional cause of death is the co-existent tendency to excessive bleeding. This is not often spontaneous bleeding, and attempts to demonstrate a consistent defect in hemostasis have yielded equivocal results. This would lead the casual observer to doubt that any real hazard of bleeding exists. Nevertheless, this is a very real complication in terms of incidence and morbility.

Bleeding may be particularly troublesome because:

a) It is often unexpected in the absence of relevant family and past history.
b) It may occur only after surgery or trauma, and
c) Blood loss may be so severe that it masks the underlying disease.

Post-Operative Post-Traumatic	23%
Purpura	20%
Gastro-Intestinal	9%
Menorrhagia	9%
Epistaxis	3%

Figure 1
Site of bleeding in polycythemia.

A review of several large series indicates an incidence of hemorrhagic complications as high as 30%[1-4]. While this is often limited to post-traumatic hemorrhage, spontaneous bleeding does occur. The site of bleeding is variable as indicated in fig. 1. Purpuric and ecchymotic lesions may be quite extensive[5] as shown in fig. 2.

---

*Presented in part at the Eighth Annual Michigan Regional Meeting of the American College of Physicians.
†Division of Hematology.
‡Division of Coagulation. Present address: Dept. of Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin.
§Division of Coagulation. Present address: Dept. of Physiological Pharmacology, College of Medicine, Wayne State University, Detroit, Michigan.
¶Division of Coagulation.

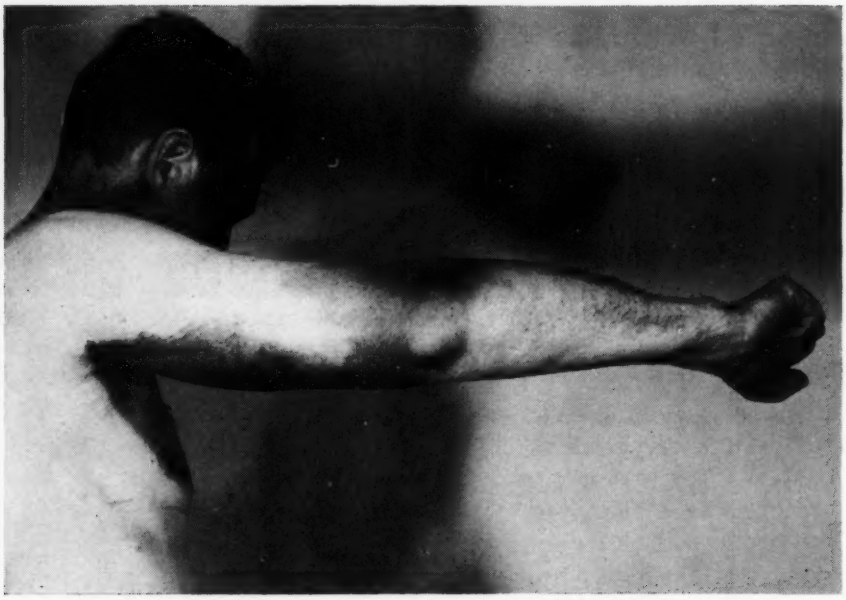


Figure 2
Extensive hematoma of the arm — an initial symptom in this patient.

MATERIALS AND METHODS

Thirty patients with polycythemia vera who have been treated regularly at the Henry Ford Hospital were studied. This was a representative group, including patients with treated, untreated and spent polycythemia. Their records were reviewed in order to determine the nature of hemorrhage in this disorder — its incidence, severity and duration, as well as the precipitating factors. After clinical evaluation, these patients had a hemostatic survey which included conventional bleeding time, coagulation time, tourniquet test, prothrombin time, prothrombin consumption, fibrinogen determination, and plasma clotting time. In addition, isolated platelets of these patients were assayed for factor 3 activity. Also, detailed and quantitative observations were made of the abnormal behavior of the polycythemic clot.

Abnormalities in the rate of clot retraction,[6] the electrolytic resistance of the retracting clot,[7] and the retention of red cells by the clot[8] have been previously noted. In vitro, the clot is typically of small volume, and most of the red cells are lost from the clot, falling to the bottom of the test tube (fig. 3) We have devised a quantitative measure of the loss of red cells from the retracting clot — the "fall-out" phenomenon. In essence this involves a quantitative determination of the hemoglobin lost by the clot from a measured volume of blood. The latter may then be expressed as a per cent of the whole blood hemoglobin. In forty normal controls this value was in the range of 20-40%.

RESULTS AND COMMENTS

Clinically significant bleeding occurred at some time during their course in 36% of these patients. The sites of bleeding are indicated in fig. 1. Whole conventional studies of the hemostitic mechanism in polycythemia vera returned normal values, this study revealed consistent deficiencies in platelet factor 3 activity and in the platelet-fibrin network of the clot. The former was reflected in the factor 3 assay of polycythemic platelets (fig. 4) and in the thromboplastin generation test. The latter was reflected in the excessive fall-out value of polycythemic clots. The loss of red cells from the clot consistently exceeded 45%, and was often in the range of 60-95%.

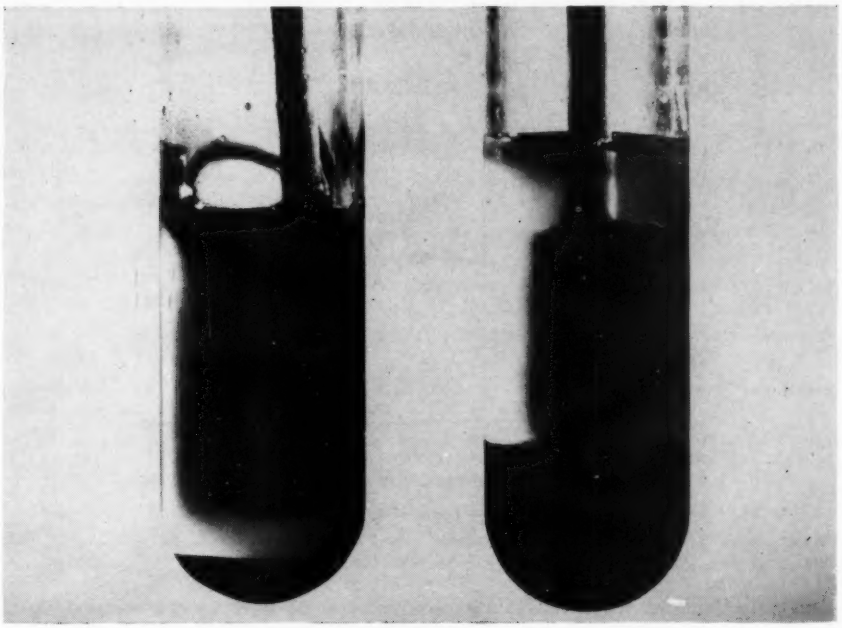


Figure 3

The "fall-out" phenomenon. The polycythemic clot (right) has lost much of the red cell mass. Control is on the left.

This excessive fall-out was apparent in patients with polycythemia vera who had manifest thrombocytosis at some time during their course. It was reduced by treatment, but was not abolished even when hematocrit and hemoglobin values were reduced to subnormal values. Thrombocytoses due to other cause, and moderate elevations of hemoglobin due to other forms of erythrocytosis were not reflected in excessive fall-out, suggesting that the test may have some diagnostic screening value in relation to polycythemia vera. However, very marked elevations of hemoglobin (in excess of 20 gms.) in secondary polycythemia were associated with excessive loss of red cells from the clot. The test is of greatest value prior to treatment in that the fall-out is often reduced temporarily by venesection or radioactive phosphorus treatment.

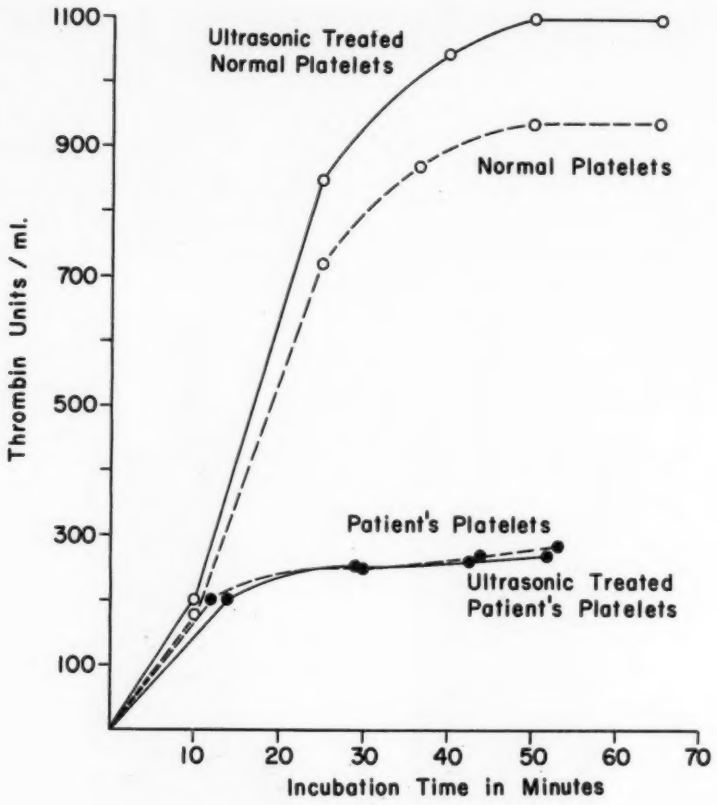


Figure 4

Platelet factor 3 assay. This activity of normal platelets is shown in contrast to the subnormal activity of patient's (polycythemic) platelets.

The previously noted defects are probably not the only factors contributing to excessive bleeding in polycythemia vera. a) Physical distention of the vascular system is a plausible cause of bleeding. b) Some observers have presented evidence that intravascular coagulation depletes prothrombin and fibrinogen to a point where bleeding occurs.[9] c) The anticoagulant effect of excessive numbers of platelets has been confirmed by Spaet, Bauer and Melamed.[10] d) On rare occasion, fibrinolytic activity has been noted in polycythemia vera.[11] e) Preliminary results with the partial thromboplastin test[12] in some of our patients have returned prolonged values, suggesting that the plasma of polycythemic patients may be less responsive to thromboplastin than is normal plasma.

Among the practical conclusions are the following: 1) Polycythemia should be suspected in bleeding patients whose conventional studies appear to be normal. 2) Treatment is of value in preventing hemorrhage as well as thrombosis. It is true that both complications occur in treated or controlled patients, but the incidence is

considerably reduced by treatment. 3) Caution in the use of anticoagulants in these patients is of vital importance. 4) Elective surgery in the untreated patient should be deferred until control has been established with radioactive phosphorus. Emergency surgery should be preceded, if feasible, by reduction of blood counts to normal range by phlebotomy.

SUMMARY

The incidence and nature of hemorrhage in polycythemia vera has been reviewed. A functional defect in the polycythemic platelet has been demonstrated, and the aberrant behavior of the clot has been confirmed. The latter has been studied in detail and quantitatively determined. The clinical application of some of these findings in the management of patients with this disorder has been briefly outlined.

REFERENCES

1. Lawrence, J. H., Berlin, N. I., and Huff, R. L.: Nature and treatment of polycythemia; studies on 263 patients, Medicine 32:323, 1953.

2. Wasserman, L. R.: Polycythemia vera — its course and teratment: Relation to myeloid metaplasia and leukemia, Bull. New York Acad. Med. 3:343, 1954.

3. Tinney, W. S., and others: Hematologic complications of polycythemia vera, Proc. Staff Meet., Mayo Clin. 18:227, 1943.

4. Norman, I. L., and Allen, E. V.: Vascular complications of polycythemia, Am. Heart J. 13:257, 1937.

5. Whitelaw, D. M., and Thomas, G. C.: Massive subcutaneous hemorrhage in polycythemia vera, Canad. M.A.J. 72:128, 1955.

6. Rosenthal, R. L.: Blood coagulation in leukemia and polycythemia; value of the heparin clotting time and clot retraction rate, J. Lab. & Clin. Med. 34:321, 1949.

7. Henstell, H. H., Henstell, I. S., and Ornitz, E.: Electrolytic resistance of the blood clot in polycythemia before and after radiation and its relationship to clot retraction, Am. J. Clin. Path. 21:820, 1951.

8. Wilson, S. J., Heath, H. E., and Larsen, W. E.: Polycythemia vera; study of coagulation abnormalities, both thrombotic and hemorrhagic, J. Kansas M. Soc. 40:90, 1958.

9. Levin, W. C., and others: Mechanism of hemorrhagic manifestations associated with thrombocythemia, J. Lab. & Clin. Med. 46:930, 1955.

10. Spaet, T. H., Bauer, S., and Melamed, S.: Hemorrhagic thrombocythemia; blood coagulation disorder, A.M.A. Arch. Int. Med. 98:377, 1956.

11. Björkman, S. E.: Three cases of polycythemia with fibrinopenia, Acta med. Scand. 129:472, 1948.

12. Langdell, R. D., Wagner, R. H., Brinkhous, K. M.: Effect of antihemophilic factor on one-stage clotting tests, J. Lab. & Clin. Med. 41:637, 1953.

COMPARATIVE STUDY OF PENICILLIN G AND TWO SYNTHETIC PENICILLINS

JOSEPH P. TRUANT, Ph.D.*

Penicillin has been commercially available for almost two decades. During this period its usefulness and limitations have been well established. The gradual appearance of resistant strains of *staphylococcus aureus* and the increasing number of serious allergic manifestations presently represent the chief drawbacks to its efficient and widespread use.

Until recently penicillin compounds could be produced only by means of biological fermentation procedures thus necessarily limiting the type as well as the number of compounds that could be prepared. In 1957, Sheehan and Henery-Logan[1] succeeded in accomplishing the first synthesis of a natural as well as related penicillins. Batchelor *et al*[2] gave further impetus to the synthesis and evaluation of numerous new and different compounds when they isolated 6-amino-penicillanic acid in 1959. This recent disclosure prompted Perron *et al*[3] to synthesize a variety of novel penicillins not amenable to production by biosynthetic methods.[4] One of the many new penicillins[5] which has shown some promise was potassium a-phenoxyethyl penicillin (phenethicillin). This compound is presently being marketed under the following trade names: alpen, chemipen, darcil, maxipen, syncillin, etc.

Since its development, potassium a-phenoxyethyl penicillin has been studied extensively by microbiological[6] and pharmacological[7] methods. These reports include clinical studies on absorption, excretion, toxicity, tolerance, and efficacy.

Another new and unique synthetic penicillin which shows considerable promise against resistant staphylococci is sodium dimethoxyphenyl penicillin. This new product was initially studied by several English investigators,[8-9] who showed that the drug was effective against the resistant staphylococci. This penicillin is being marketed in this country by Bristol-Laboratories under the trade name of staphcillin.

Comparative studies of pencillins G,V, a-phenoxyethyl and dimethoxyphenyl have shown that different penicillins show quite large differences in the range of antibacterial activity. The present paper describes our laboratory studies using three of these penicllins.

Materials and Methods:

Two hundred and twenty-four strains of coagulase-positive staphylococci were isolated from clinical specimens received by our bacteriology laboratory from September to December 1960. The organisms were isolated from numerous sources (blood, sputum, throat, wound infections, etc.). The determinations of minimum inhibitory concentrations were performed by means of three procedures:

1. disk method using penassay (Difco)* and trypticase soy agar with BBL disks.**
2. tube dilution technique using penassay broth (Difco).*
3. cup-plate assay-Penassay agar (Difco).*

*Department of Laboratories.

*Difco Laboratories, Detroit, Michigan.
**BBL disks are produced by Baltimore Biological Laboratory, Baltimore, Maryland.

The disk concentrations were as follows:

(a) Penicillin G — 2 and 10 units.

(b) a-phenoxyethyl — 2 and 15 units.

(c) dimethoxyphenyl — no disks available.

Dilutions of all three penicillins for the tube dilution series were prepared in penassay broth (Difco)* at the following concentrations per ml. — 0.1, 1.0, 2.0, 10, 20 and 50 units.

RESULTS

The initial studies (Table 1) using 30 strains of staphylococci obtained at random showed that six or twenty per cent of the organisms were more sensitive to disks containing a-phenoxyethyl penicillin than to those containing penicillin G. However. it should be noted (see table 1) that the "high concentration disks" varied in the amount of each drug which they contained. The penicillin G disks were 10 units whereas the a-phenoxyethyl penicillin disks contained 15 units of this drug.

TABLE I

Disk Tests using penicillin G (10 units) and a-phenoxyethyl penicillin (15 units) against 30 strains of *S. aureus.*

	Penicillin G	a-phenoxyethyl penicillin
Sensitive strains	19	25
Resistant strains	11	5

Further experiments using both low (2 units) disks and high concentrations disks (10 or 15 units) with 124 strains of *staphylococcus aureus* (see table 2) confirmed the initial results—namely that a-phenoxyethyl penicillin showed greater activity than did penicillin G.

TABLE II

Summary of 124 strains of *S. aureus* tested with penicillin G. and a-phenoxyethyl penicillin disks.

Degrees of susceptibility*	V. S.	M. S.	S. S.	SENSITIVE**
Sensitive to a-phenoxyethyl but resistant to Pen. G	1(0.8%)	3(2.5%)	28(23%)	32(26%)
Sensitive to both Penicillins				
—Pen. G.	45(36%)	4(3%)	6(5%)	55(44%)
—a-phenoxyethyl	47(38%)	8(6%)	None	55(44%)

*V. S. Significant zone of inhibition around both disks.
M. S. Small zone surrounding low disk and large zone around high disks.
S. S. Zone of inhibition around high disk only.
**Total number of all strains showing some degree of susceptibility to the respective compounds.

The second series of experiments employing similar disks gave sufficient indication that a-phenoxyethyl penicillin was more inhibitory than penicillin G (see tables 2, 3). However, it should be emphasized that in many instances (23% of the strains) the a-phenoxyethyl penicillin was only slightly more inhibitory than penicillin G. Therefore, it was necessary to determine the minimum inhitory concentration (MIC)

of each compound for the individual strains in order to better compare the *in vitro* activity of the two penicillins. The data in Table 4, shows a cross-section of the tube dilution results obtained with the penicillins discussed above. The results demonstrate that 6 of the 10 strains were susceptible to lower concentrations of a-phenoxyethyl than to penicillin G, thus supporting the data obtained during the preliminary studies employing the disk procedure.

TABLE III

Comparative study of the three penicillins using 17 strains of *S. aureus.*

STRAIN NO.	PEN. G.	a-phenoxyethyl	dimethoxyphenyl
3-7, 13, 15	VS	VS	VS
8	SS	MS	VS
9, 14	R	SS	VS
10	R	R	VS
11	R	MS	VS
12	MS	MS	VS
15	VS	VS	VS
16	R	R	R
29, 30	R	R	VS

The third series of experiments consisted only of tube dilution and cup-plate assays of dimethoxyphenyl penicillin since disks of this penicillin were not available. A preliminary survey of 35 strains of *S. aureus* isolated consecutively and without selection from patients showed that there were some important differences between this penicillin and the two other penicillins. All of the strains resistant to penicillin G (10-50 units) were inhibited by 10 units of sodium dimethoxyphenyl penicillin (see table 3). The most important finding was the remarkable uniformity of all our strains (except one) to 10 units of the latter. Irrespective of their sensitivity to penicillin G or a-phenoxyethyl penicillin, 223 or 224 strains were inhibited by 2-10 units (1-7 mcg) per ml., of sodium dimethoxyphenyl penicillin. Many of the strains tested were resistant to relatively high concentrations (50 units per ml. or more) of penicillin G or a-phenoxyethyl penicillin as well as to other antibiotics such as chloromycetin and the tetracyclines. Many of the strains were penicillinase producers obtained from hospital acquired infections, including many of the common bacteriophage types such as 80, 81.

The results of the cup-plate assays using the three penicillins have not been presented in tabular form since they generally agreed with the data obtained by the disk and tube dilution procedures.

TABLE IV

Tube dilution procedure comparing the effect of the 3 penicillins against 10 'wild' strains of *S. aureus*.

Identification No. of 'wild' strains	Penicillin G						a-phenoxyethyl penicillin						dimethoxyphenyl pen.					
	0.1	1.0	2.0	10	20	50	0.1	1.0	2.0	10	20	50	0.1	1.0	2.0	10	20	50
99	+	+	+	+	+	−	+	−	−	−	−	−	+	+	+	−	−	−
107	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
108	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
109	+	+	+	+	+	−	+	−	−	−	−	−	+	+	+	−	−	−
110	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−
111	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
114	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−
115	+	+	+	+	−	−	+	−	−	−	−	−	+	+	+	−	−	−
116	+	+	+	+	−	−	+	−	−	−	−	−	+	+	+	−	−	−
117	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−

*Concentration of the penicillins is expressed in units.

+ = penicillin did not inhibit growth.

− = organism inhibited by the compound.

DISCUSSION

Potassium alpha-phenoxyethyl penicillin (Phenethicillin)[10] is a new semisynthetic penicillin produced by the N-acylation of alpha-phenoxypropionic acid (the phenyl ether of lactic acid) and 6-aminopenicillanic acid. The resulting molecules contains an additional asymetric carbon atom and thus occurs in two isomeric forms. A mixture of the isomers is said to possess biologic and chemical properties superior to those of either isomer. Until 1957,[1] penicillin could be produced only by means of fermentation (biologic) procedures. In using 6-aminopenicillanic acid, the penicillin intermediate obtained by fermentation methods, it has been possible to synthesize many new penicillins.[5]

Gourevitch *et al*[6] have shown that if minimum inhibitory concentrations of a-phenoxyethyl penicillin are determined against a variety of clinically isolated resistant staphylococcal strains the inhibitory end points are invariably lower than those obtained with penicillin G or V. The results obtained in our laboratory with G agree with these findings.

Although the minimum inhibitory concentrations with a-phenoxyethyl penicillin appear to be considerably lower in some instances than those obtained with G and V, it does not follow that the enhanced sensitivity is marked enough to be therapeutically significant. Griffith[11] compared the antibiotic activity in sera after administering the three different penicillins. His findings showed that postassium V gave two to five times greater antibacterial activity in serum than potassium penicillin G when these two compounds are administered orally. When equal quantities of penicillin V and a-phenoxyethyl penicillin are given orally, the former produces earlier onset and higher antibacterial activity in sera than the latter.

Griffith[11] agrees with Jones and Finland[12] that measuring antibacterial activity in the sera using the serial dilution method and the pathogen as the indicator organism is more informative regarding antibacterial effectiveness than reporting "blood levels", in micrograms. Thus, this method is recommended as a useful tool for comparing two different antibiotics or when a new modification is compared with the older antibiotic. It should be emphasized that in so far as strains of staphylococci resistant to a-phenoxyethyl penicillin on *in vitro* testing are concerned, one might conclude that serums containing the latter are more bactericidal, but such a conclusion may not be entirely warranted. It is evident from the studies of Griffith[11] as well as Martin *et al*[13] that despite higher concentration of a-phenoxyethyl penicillin, such serums are not always more consistenly eradicative against penicillin-"sensitive" staphylococci than those containing penicillin G or V.

The *in vitro* studies conducted in our laboratory using dimethoxyphenyl penicillin against penicillin resistant staphylococci showed this drug to be effective at 1-7 mcg. per milliliter. It is the first penicillin which combines considerable antibiotic activity with almost complete resistance to the action of staphylococcal penicillinase as discussed by Knox.[9] This author has shown that dimethoxyphenyl penicillin inhibits the growth of both penicillin-sensitive and penicillin-resistant staphylococci at concentrations of from 2-4 ug per ml., however, our findings indicate a range of susceptibility of from

1 to 7 ug. per ml. The latter data is in closer agreement with the information published by Bristol-Laboratories, the manufacturers of staphcillin.*

The most important and perhaps the only significant property of sodium dimethoxyphenyl penicillin is that it is equally active *in vitro*, in concentrations of approximately 1-7 micrograms per ml., against all except occasional strains of *S aureus* regardless of their susceptibility patterns to penicillin G or a-phenoxyethyl penicillin. The British investigators have reported that dimethoxyphenyl penicillin is at least fifty times more active against penicillinase-producers than is penicillin G. This synthetic penicillin is minimally, if at all, degraded by staphylococcal penicillinase. This penicillin derivative is only about one-twentieth as active as penicillin G against Group A streptococci and *Neisseria*, about one-tenth as active against strains of pneumococci and Streptococcus viridans and inactive against enterococci and gram-negative bacilli (except *Hemophilus* which is only slightly sensitive). Thus far, its useful activity therefore appears to be limited to severe penicillin resistant staphylococcal disease.

SUMMARY

1. Potassium a-phenoxyethyl penicillin produced greater antistaphylococcal activity *in vitro* than did potassium penicillin G against many of the 224 strains tested.

2. Sodium dimethoxyphenyl penicillin is effective at concentrations of 1-7 micrograms per ml. against 223 of the 224 strains of staphylococci tested. This unique attribute is probably due to the fact that this compound is stable in the presence of staphylococcal penicillinase.

3. A comparison of the *in vitro* activity of pencillin G with the two synthetic penicillins makes it clear that different preparations of this antibiotic show significant differences in their antibacterial activity. Therefore, one is not justified in treating all penicillins as though they were equivalent.

REFERENCES

1. Sheehan, J. C. and Henery-Logan, K. R.: The total synthesis of penicillin V, J. Am. Chem. Soc. 79:1262, 1957.

2. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N.: Synthesis of penicillin: 6-aminopenicillanic acid in penicillin fermentations, Nature 183:257, 1959.

3. Perron, Y. G., Crast, L. B., Gottstein, W. F., Minor, W. F., and Cheney, L. C.: Synthesis of potassium penicillin-152 [Potassium (a-phenoxyethyl) penicillin] and both diastereoisomers. Antibiotics Annual 1959-1960, p. 107.

4. Behrens, O. K.: Biosynthesis of penicillins. In, National Academy of Sciences: Chemistry of Penicillin, Princeton, N. J., Princeton University Press, 1949, p. 657.

5. Gourevitch, A., Hunt, G. A., and Lein, J.: Structure-activity relationships in a series of synthetic penicillins, Antibiotics & Chemother. 10:121, 1960.

6. Gourevitch, A., Hunt, G. A., and Lein, J.: Microbiological studies on potassium penicillin-152 [Potassium (a-phenoxyethyl) penicillin]. Antibiotics Annual 1959-60, p. 111.

7. Pindell, M. H., Tisch, D. E., Hoekstra, J. B., and Reiffenstein, J. C.: Pharmacological studies on potassium penicillin-152 [Potassium (a-phenoxyethyl) penicillin]. Antibiotics Annual 1959-1960, p. 119.

*Staphcillin is sodium dimethoxyphenyl penicillin produced by Bristol-Laboratories, Syracuse, New York.

8. Douthwaite, A. H., and Trafford, J. A.: A new synthetic penicillin, Brit. M. J. 2:687, 1960.

9. Knox, R.: A new penicillin (BRL 1241) active against penicillin-resistant staphylococci, Brit. M. J. 2:690, 1960.

10. Morigi, E. M. E., Wheatley, W. B., and Albright, H.: Clinical and laboratory studies with potassium penicillin-152 [Potassium (a-phenoxyethyl) penicillin]: A new synthetic penicillin. Antibiotics Annual 1959-1960, p. 127.

11. Griffith, R. S.: Comparison of antibiotic activity in sera after the administration of three different penicillins, Antibiotic Med. 7:129, 1960.

12. Jones, W. F., Jr., and Finland, M.: Antibiotic combinations; antistreptococcal and antistaphylococcal activity of plasma of normal subjects after ingestion of erythromycin or penicillin or both, New England J. Med. 255:1019, 1956.

13. Martin, W. J., and Nichols, D. R.: Penicillin V and phenethicillin potassium in serum: Comparison of concentrations and of antibacterial effects, Proc. Mayo Clin. 35:577, 1960.

THE EFFECT OF SODIUM COLISTINEMETHANESULFONATE ON STRAINS OF PSEUDOMONAS

JOSEPH P. TRUANT, Ph.D.*

An antibiotic produced by the soil-borne *Bacillus polymymixa* var *colistinus* [Bacillus (aerorobacillus) colistinus] was isolated from a soil sample (Fukychima Prefecture, Japan) by Koyama *et al* in 1950.[1] The drug was given the generic name of colistin. Two forms are commercially available: (1) colistin sulfate (Coly-mycin S **) and (2) sodium colistinmethanesulfonate (Coly-mycin M **). These are complex polypeptide antibiotics which have chemical properties related to those of polymyxin[2]. Shidlovsky *et al*[1] have stated that the principle amino acids of colistin are as follows: 1-threonine, d-1-leucine and 1-alpha, gamma-di-aminobutyric acid but it does not contain either d-serine or phenylalanine.

Sodium colistinmethanesulfonate and colistin sulfate are said to have marked bacteriostitic and bactericidal activity *in vitro* against such groups of gram negative organisms as *Pseudomonas, Salmonella, Shigella, Escherichia* and others.[2] The compounds apparently have less activity against gram positive bacteria and fungi.

During the last decade the clinical picture of infectious disease has undergone a rapid and rather dramatic change from a position of immediate concern for such virulent organisms as the pneumococci and streptococci to one of minor clinical importance for these pathogens. The rise to a position of importance by organisms formerly considered as saphrophytes or minor pathogens is well illustrated by the fact that organisms such as *Aerobacter, Escherichia, Proteus* and *Pseudomonas* are being found with increasing frequency in infections (i.e. urinary tract). *Pseudomonas* is commonly found in refractory infections. It is frequently found when foreign bodies have been introduced or if there are deficient host resistance factors associated with calculi, urinary tract obstruction, drains, catheters, metastatic carcinomas, etc. Furthermore, it should be understood that treatment with chemotherapy of any type, frequently fails unless the underlying cause for the infection is removed — i.e. obstruction in urinary tract infections.

The present paper will be primarily concerned with the *in vitro* effect of colistin on clinical isolates of the genus *Pseudomonas.*

MATERIALS AND METHODS

The antibiotic was supplied in vials containing sodium colistinmethanesulfonate (coly-mycin-M). Each vial contained 150 mg. of colistin base as sodium colistinmethanesulfonate. One milligram of the pure colimycin base contains 30,000 units.

*Department of Laboratories, Henry Ford Hospital, Detroit, Michigan.

**Coly-mycin-S and Coly-mycin-M were supplied by Warner-Lambert Research Institute, Morris Plain, New Jersey.

The concentrations of colistin in this report have been expressed throughout as follows: 1, 2, 10, 20, 50 and 100 micrograms (ug) of colimycin base per milliliter (ml.) of diluent for the tube dilution as well as the cup-plate procedures. The lyophilized antibiotic was reconstituted with phosphate buffer (Ph 7.3). This compound was ordinarily reconstituted and used on the same day. Commercially available preparations of the other Chemotherapeutic agents were supplied by Mr. Fink of the Chas. Pfizer and Company.*

The organisms tested were recent isolates from patients, whose specimens were submitted to our bacteriology laboratory. Twenty-eight recently isolated strains of *Pseudomonas* were examined.

The organisms were isolated on sheep's blood trypticase soy agar plates which were also used for performing disk susceptibility tests with all chemotherapeutic agents except the sulfonamide drugs. Tube dilution and cup-plate assay studies were performed using Bacto-S-R** broth and agar respectively.

Bacto-S-R agar was also used for the susceptibility tests employing sulfonamide disks. The tests with the latter were read after 18 hours incubation at 37° C. The results of the tube dilution were recorded at 24, 48, and 72 hours after incubation at 37° C. The size of the zone of inhibition was measured with a caliper and recorded in millimeters (mm.).

The inoculum for the tube dilutions consisted of 0.1 ml. of a 4-hour culture inoculated into 10 ml. of broth containing the antibiotics. The plate counts which were prepared to determine inoculum size showed approximately 25 million cells per ml. of 4-hour culture. Therefore, each tube was inoculated with approximately 2,500,000 cells of *Pseudomonas.*

RESULTS

The *in vitro* spectra of sodium colistinmethanesulfonate as well as other chemotherapeutic agents[3] were determined using 28 recently isolated strains of *Pseudomonas.* The minimum inhibitory concentrations (MIC) obtained by the tube dilution technique against some of the strains are shown in Table 1. The organisms designated in this table represent a cross section of the low, mean and high inhibitory concentrations (M.I.C.) obtained with 10 of the 28 strains of *Pseudomonas.*

The results of the tube dilution series (Table 1) show that the majority of strains were sensitive to 20 micrograms of coly-mycin-M per ml. A few such as strain no. 7 and 18 were resistant to 20 and 50 ug. per ml. respectively. Inspection of the table 1 and 2 will show that the cup-plate assay results compare favorably with the values obtained with the tube dilution technique. The MIC figures in Table 2 further serve to show very excellent correlation between the two procedures except for an occasional strain (i.e. no. 6.).

*Chas. Pfizer and Company, Inc. Brooklyn 6, N. Y.
**Bacto-S-R medium was obtained from Difco Laboratories, Detroit.

TABLE 1

A cross-section of *Pseudomonas* strains and their MIC values using coly-mycin-M.

	Tube dilution method						Cup-plate procedure					
	1 ug*	2	10	20	50	100	1 ug*	2	10	20	50	100
Strain no. 1	+**	+	+	−	−	−	+	+	+	o 10 mm.	15	16
2	+	+	−	−	−	−	+	+	9	12	17	18
3	+	+	−	−	−	−	+	+	+	9	12	15
4	+	+	+	−	−	−	+	+	+	10	12	15
7	+	+	+	+	−	−	+	+	+	9	12	13
11	+	+	+	−	−	−	+	+	+	10	12	14
14	+	+	−	−	−	−	+	+	10	14	17	18
17	+	+	+	−	−	−	+	+	+	+	10	11
18	+	+	+	+	+	−	+	+	+	10	10	13
23	+	+	+	−	−	−	+	+	+	10	14	16

*Concentration of sodium colistinmethanesulfonate is expressed in micrograms (ug) per milliliter.

**

+ = growth of organisms − no inhibitory effect.

− = no growth of organisms − inhibitory effect.

0 = numbers refer to the size of the zone of inhibition in millimeters (mm.). The stainless steel cylinders are 8 mm. in diameter.

TABLE 2

Minimum inhibitory concentrations (MIC) of sodium colistinemethanesulfonate using a cross-section of *Pseudomonas* strains.

Strain no.	Tube dilution method	Cup-plate procedure
5	>2 ug*	>2 ug*
6	2	20
8	10	10
9	10	10
10	2	10
12	2	2
15	20	20
24	10	10
27	2	10
28	10	10

*The MIC values are greater than the values shown in the table but less than the next largest tube dilution or cup-plate value shown in Table 1. ie. The MIC for strain #5 is between 2 and 10 ug. since 2 ug. did not inhibit growth whereas 10 ug. produced inhibition.

Experience with the determination of MIC values against a variety of organisms has shown that titration end points with Coly-mycin-M and in fact with many other antibiotics depend on such conditions as (a) size of inoculum, (b) type of media, (c) effect of Ph. etc. For example Swartz *et al*[2] have studied the *in vitro* activity of colistin using various sizes of inoculum. They demonstrated that if the inoculum size varied from 10^3 to 10^8 the MIC values for sodium colistinemethanesulfonate would vary from 0.03 to 10.0 ug per millimeter respectively. Since the inoculum size and media used for the tube dilution and cup-plate procedures was not exactly similar with those of Swartz *et al*,[2] these may be possible reasons for the variation. The same investigators reported that sodium colistinemethanesulfonate was up to 10 times more active at Ph 6.0 than at 8.0 when evaluated in terms of the size of the inhibition zone. The media (broth and agar) used in our series of experiments had a Ph range of 7.3 to 7.4. Therefore, it is unlikely that the PH factor contributed significantly to the occasional differences in MIC values obtained with the two techniques.

Another aspect considered in this series of experiments was the antibacterial action of colistin. Our experiences has shown that the antibacterial activity of the drug is primarily bacteriostatic rather than bactericidal. The bactericidal activity was demonstrated by the *in vitro* technique of subculturing from the tubes that showed no growth in the broth dilution series. The bacteriostatic and bactericidal concentrations, for the sensitive organisms, were different in 66% of the series tested.

The results with colistin also essentially agree with the information published by several investigators who have studied the properties of poly-myxin.[4,5,6] Repeated tests with these agents, using similar strains, showed slight but not significant variations in the bactericidal concentrations for many of the organisms; however, there were several exceptions. Sodium colistinmethanesulfonate and polymyxin B were generally more effective bactericidal agents than dihydrostreptomycin. The data also demonstrated that dihydrostreptomycin resistant strains of *Pseudomonas* may be sensitive to either colistin or polymyxin B.

DISCUSSION

Several investigators[3,7,8,9] have studied the *in vitro* activity of the broad spectrum antibiotics (chloramphenicol, tetracycline, etc.) as well as the sulfonamides (sulfanilamide, sulfapyridine) using a large variety of *Pseudomonas* isolates. In general, the reports have shown a steady increase in the incidence of organisms which have developed resistance to these chemotherapeutic agents. As a result, clinicians have frequently had no other choice than to use the relatively more toxic antibiotics—i.e. bacitracin, neomycin and polymyxin. These agents have proven to be successful in the treatment of some *Pseudomonas* infections, especially those resistant to the common therapeutic agents. The studies of *Schwartz et al*[2] and *Roberts et al*[10] indicate that colistin sulfate and sodium colistinemethanesulfonate may possibly fill an important therapeutic need in this clinical area.

More recently colistin has been considered as an antiseptic for preparation of the bowel previous to surgery: absorption of colistin from the gastrointestinal tract following oral administration is negligible, a characteristic which precludes its effective-

ness, by this route, for systemic infections but is advantageous when the antibiotic is employed for the purpose of sterilization of the large bowel.[1] Shidlovsky *et al*[1] disclosed that 400 mg. of colistin administered orally in one day reduced the total amount of gram negative organisms to a greater extent than did the smaller dosage of 200 mg. They suggested that 2 grams erythromycin propionate be given in four equal divided doses in one day as well as 400 mg. of colistin in order to give a greater and more consistent reduction of the gram positive flora. The authors did not observe any untoward reactions in any of the 21 patients during the course of treatment and the observation period.

ACKNOWLEDGMENT

The author wishes to express his appreciation to William Penn for his technical assistance.

SUMMARY

1. Sodium colistinemethanesulfonate showed antibacterial activity *in vitro* against strains of *Pseudomonas*.

2. The drug demonstrated bactericidal as well as bacteriostatic activity *in vitro*.

3. Sodium colistinemethanesulfonate was slightly less active than polymyxin B against the strains which were examined.

REFERENCES

1. Shidlovsky, B. A., Fetzer, V., and Prigot, A.: The effect of colistin alone and in combination with erythromycin propionate on the intestinal flora of man, Antibiotics & Chemother. 10:640, 1960.

2. Schwartz, B. S., Warren, M. R., Barkley, F. A., and Landis, L.: Microbiological and pharmacological studies of colistin sulfate and sodium colistinemethanesulfonate. Antibiotics Annual 1959-1960, p. 41.

3. Truant, J. P.: *In vitro* evaluation of the commonly used antibiotics against *Pseudomonas* isolates. 1st Conference on Antimicrobial Agents, Society of Industrial Microbiology, Washington, D. C., 1960.

4. Brownlee, G., and Bushby, S. R. M.: Chemotherapy and pharmacology of aerosporin; a selective gram-negative antibiotic, Lancet 1:127, 1948.

5. Bliss, E. A., Chandler, C. A., and Schoenbach, E. B.: *In vitro* studies of polymyxin, Ann. New York Acad. Sc. 51:944, 1949.

6. White, H. J., Alverson, C. M., Baker, M. J., and Jackson, E. R.: Comparative biological studies of polymyxin and "aerosporin", Ann. New York Acad. Sc. 51:879, 1949.

7. Kass, E. H.: Chemotherapeutic and antibiotic drugs in the management of infections of the urinary tract, Am. J. Med. 18:764, 1955.

8. Dowling, H. F., Lepper, M. H., and Jackson, G. G.: Clinical significance of antibiotic-resistant bacteria, J.A.M.A. 157:327, 1955.

9. Bryer, M. S.: The chemotherapy of bacterial infections refractory to the common antibiotics, Am. J. Med. 18:782, 1955.

10. Roberts, E., Kuharic, H. A., and Kirby, W. M.: Clinical appraisal of colistin. 1st Conference on Antimicrobial Agents, Society of Industrial Microbiology, Washington, D. C., 1960.

THE NEUROPHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM; A SIMPLIFIED REVIEW

ROBERT R. SCHOPBACH, M.D.* AND KEITH B. AKINS, M.D*.

Studies concerning the mode of action of the nervous system are daily producing new information. Because different animals may respond differently and because the response varies widely under only slightly different experimental conditions, the results may be quite contradictory. With present incomplete and imperfect knowledge more than one hypothesis can explain the action and who can say which is correct. With this in mind one viewpoint is presented here to clarify the rationale behind current neuropharmacology.[1]

Behavior was formerly considered to result from sensory stimuli going to discrete cortical sensory areas and thence evoking responses from other discrete motor areas with a few checks and balances furnished by associated areas. Today studies indicate that the brain is a most complex network of feedback, inhibitory, and amplifying circuits acting as a unit; each must function properly if the optimum response is to be made. With the aid of almost incredible technical devices such as pipettes and electrodes placed within single cells our dim understanding of these functions is rapidly increasing and altering former hypotheses. A functional balance peripherally between the sympathetic and the parasympathetic systems is still considered valid. A similar balance has been postulated for subcortical portions of the central nervous system; the components are called ergotrophic and trophotrophic.[2] The ergotrophic system is integrated with the sympathetic system to effect arousal and increased muscular and psychic activity while the trophotrophic system tends to return the body to a state of equilibrium, inactivity, decreased muscular tone, and even sleep. The ergotrophic system acting with the peripheral sympathetic system deals with fight or flight and similarly the trophotrophic with the maintenance of the specie through its own metabolism and through reproduction. The activity of each of these systems is effected by corresponding neurohumors; nor-epinephrine and acetyl choline[3] peripherally and nor-epinephrine[4] and serotonin[5] centrally respectively. Although some believe that serotonin merely modifies the actions of the nor-epinephrine rather than being a separate transmitter substance,[6] this is not the most common opinion. This paper will review primarily the activity of the central nervous system.

Coordinated cellular activity depends to a great degree upon synaptic transmission.[7,8] Such transmission is accomplished as follows: neurohumors are stored in the terminal axone buttons bound to a protein.[9] Upon the arrival of electrical impulses this bound form is split releasing the neurohumor which escapes through pores in the cell membrane into the intercellular fluid. (Diag. I) Under resting conditions this free form is released so slowly that the enzymes in the intercellular fluid and responsible for its degradation inactivate it before it has had an opportunity to affect the post-synaptic cell's membrane. When greater amounts are freed, the concentration immediately around the terminal button is so great that for a millisecond or so the enzyme is incapable of deactivating all of it and it is able to affect the post-synaptic membrane.

*Department of Psychiatry.

DIAGRAM I

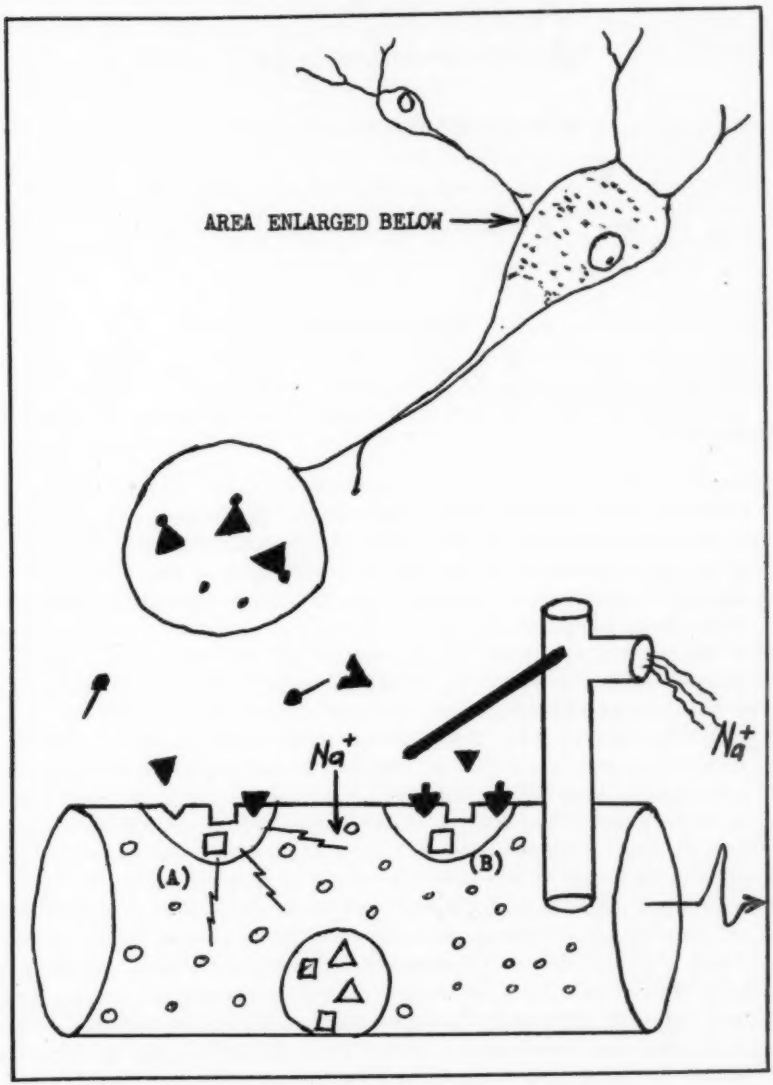
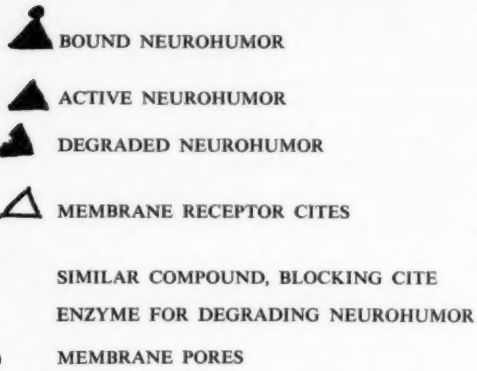


DIAGRAM I

SYMBOLS



EXPLANATION

At (A) the neurohumor is entering the receptor cites and initiating depolarization. This permits Na+ to rapidly enter and to propagate a spike discharge (at right). At (B) a compound of similar spatial configuration has blocked the cites. In the intercellular fluid the enzyme has degraded some of the neurohumor.

Certain portions of the receptor or post-synaptic cell's membrane which lie in close proximity to the terminal axone endings of the pre-synaptic cell are especially sensitive to chemical, thermal, or mechanical changes but are relatively insensitive to electrical changes. Alterations in the chemical constitution of the intercellular fluid, especially in the neurohumoral concentrations, selectively alter the permeability of the cell membrane to specific ions, especially Na^+, K^+, and Cl^- and thereby alter its electrical potential. If these changes increase the cell membrane potential, no spike discharge is initiated, in fact before any discharge could subsequently be elicited an even greater degree of depolarization than usual would have to occur. This is called hyperpolarization and results in inhibition of action. Other stimuli produce a depolarization (Diag. IA) and initiate an alteration in the electric field which spreads to the surrounding areas of the membrane which are sensitive to such electrical changes. Through the continued action of one receptor area or through the summation of the actions from a number of such areas a characteristic electro-chemical spike discharge is elicited. This discharge is due in part to the almost explosive speed by which the sodium ion reaches equilibrium once the membrane's selective permeability is altered. The electrical discharge however antedates the sodium ion transport and may be due to the splitting of a lipo-protein in the cell membrane. Following such a discharge the cell must be given time for its inherent metabolic sodium pump to re-establish the ionic differentials in which the sodium ion concentration is lower and the potassium ion concentration is higher within the cell than in the extracellular fluid. Should the chemically responsive portion of the post-synaptic cell membrane be constantly stimulated, it would constantly produce depolarizing currents which would prevent the re-establishment of these differentials and the cell would be unable to generate the potential to fire again. This is known as depolarizing blockade.

From this it can be seen that the response is dependent upon the concentration of the neurohumor. If there is too little, the cell will not be stimulated into action and the opposing system will assume functional predominance. If the correct amount is present, a normal balance will obtain. If the amount is increased moderately, the cell will fire more often and that system will predominate. Finally if there is too much of the neurohumor acting upon the post-synaptic membrane, it will fire once but then be unable to fire again and the opposing system will predominate. The many methods of altering synaptic transimission will be discussed later.

The energy necessary for the maintenance of the metabolic sodium pump is derived primarily from pyruvic acid-adenosine triphosphate metabolism[10] although under special conditions it can also be obtained from the metabolism of certain amino acid compounds.[11] If these energy systems are impaired, the proper ionic differential and membrane potential will not be attained and the cell will not be capable of normal activity. The enzymes which control these metabolic processes are believed to be contained in the mitochondria.[12,13] Many drugs as well as asphyxia act by impairing the functions of the mitochondria thereby reducing the overall functional capacity of the cells. GABA (γ-amino butyric acid) is closely concerned with neuronal activity and transmission. It has been considered to be another neurohumor but current neurochemical studies suggest a predominantly metabolic function.[14] That its concentration is decreased in convulsive states and increased by some anticonvulsive drugs suggests it has an inhibitory function.

In those cases in which the spike discharge must traverse a sheathed axone, any alteration in the lipids which form the sheath will interfere with the transmission especially at the internodal regions.[15] Finally with the arrival of an adequate impulse or series of impulses at the axonal dendritic buttons the entire process is recommenced.

The activity of this transmitting system can be altered at any level. The amount of the neurohumors cannot readily be increased by their administration orally or even intravenously as they do not readily cross the blood-brain barrier. However their precursors in the form of DOPA (dihydroxy phenyl alanine) for NE[16] and 5 hydroxy tryptophane for serotonin do cross this barrier and cause increased activity of their respective systems.[17] This effect becomes even more marked if an MAO inhibitor (v.i.) has been previously given. The storage of bound-form is altered greatly by trace metals and even altered Ca^{++}. The storage of bound neurohumors may be prevented by reserpine. This releases both serotonin and nor-epinephrine.[18] Initially these may cause a transitory period of increased activity. Subsequently the serotonin is reformed about fifty times as rapidly as the nor-epinephrine[19] so that the trophotrophic system is stimulated much more than the ergotrophic. The results in decreased motor and psychic activity to the point of somnolence and sometimes depression. Other cholinergic actions such as increased nasal secretion or bowel activity may provide annoying side effects. Reserpine also prevents the storage of NE in peripheral sympathetic nerves to further decrease the motor activity and, with the bradycardia, to produce hypotension. By choosing others of the reserpine analogues it is possible to obtain either the central or peripheral effects separately.[20,21] Certain other benzoquinolizine and reserpine derivatives are also capable to affecting separately

NE or serotonin. Under slightly different experimental conditions completely opposite results have been obtained in an attempt to determine which neurohumor is responsible for the sedative effects.[22,23] So the answer is as yet far from clear.

The neurohumors must pass from the axone to the receptor membrane in a great enough concentration to propagate an electrical pulse. During this passage they are being metabolized by specific enzymes especially mono-amine oxidases but also methyl transferases. The amounts of these enzymes available to act on the neurohumors may be decreased by the presence of other compounds of similar structure which "compete" for the enzymes.[24] If the $-NH_2$ is not in the terminal position, the enzyme is removed from further action. These compounds may also have some stimulatory action of their own to further increase their effectiveness. This is felt to explain the actions of amphetamines. Many CNS depressants decrease the rate of release of bound serotonin[25] but this is not felt to be the cause of the depression (sedation); their depression of cellular metabolism is considered the more important action.

If the enzyme's activity is blocked, the neurohumors will not be destroyed but will accumulate. Mono-amine oxidase is the enzyme which normally degrades both NE and serotonin; its inhibition leads to accumulations of both of these. However nor-epinephrine is additionally much more susceptible to degradation through a secondary pathway by methyl transferase than is serotonin.[26,27] This results is marked increases in serotonin and lesser increases in NE. One possible explanation is that the serotonin level rises to such a point as to "flood" the synapses and to create a depolarized blockade of the trophotrophic system. In our exprience there has been a transient period of psychic impairment a few hours after taking the MAO-inhibitor which might indicate a point where the serotonin was stimulating the trophotrophic system into temporary predominance. Although the psyche is subsequently stimulated suggesting ergotrophic domination centrally, peripherally there are signs of parasympathetic dominance especially in the genitourinary tracts.[30] Clinically MAO inhibitors were being tried to inhibit the metabolism of tubercle bacilli when it was noted that an euphoria often occurred.[28] This antidepressant action is now their chief indication although it is not certain that the improved mood is the result of either serotonin or NE; there are many other enzymes and amines which might possibly be involved.[29]

The combination of reserpine with MAO inhibitors produces opposite effects depending upon which is given first. Should the reserpine be given first, the MAO-inhibitor has no effect and sedation persists. However, should the MAO inhibitor be given first, the neurohumors released by the reserpine are not destroyed but reach such high concentrations that hyperactivity and even convulsions may occurr.[31] This could be due to stimulation by NE or flooding by serotonin. The former may be true as phenothiazines which interfere with NE block this reaction.

The receptor areas of the post synaptic cells' membrane offer intriguing possibilities for chemical activity. It can be pictured as a layer of cylindrical macromolecules with varying size pores.[31] Many drugs are of such small molecular size that they can slip through these pores and enter into the cell where they interfere with some of

the mitochondrial metabolic processes to have a very general sedative effect. This is the action of most sedatives[10] and one of the actions of phenothiazines.[33] If the drug molecules are larger, they must be of the correct spatial configuration to fit into the pore; such drugs have much more specific effects as they fit relatively few pores. Yohimbine, harman, lysergic acid, adrenochrome, and adrenolutin are all structurally related to serotonin and nor-epinephrine and might occupy their receptor sites but, being different, would produce an altered response (Diag. IB). For normal mentation the total sensory input must lie within a certain range. This varies from individual to individual. But, if it is increased too much, even the "strong" have their breaking point as was observed under prolonged stressful conditions as in World War II.[34] The opposite extreme, isolation with as complete sensory deprivation as possible, also gives rise to abnormal mental processes.[35] Thus it is not suprising that the alteration in responsivity produced by these related compounds produces disturbances in mental processes. Perhaps by understanding these "model psychoses" we can gradually understand the naturally occuring psychoses. One current postulate suggests a disturbance in epinephrine metabolism with the production of adrenochrome to be one of the causes of schizophrenia[36,37] but this is the subject of much argument. The research to prove or disprove this point adds to our general knowledge bit by bit. Such increases in neurophysiologic knowledge have permitted great advances in neuropharmacology in recent years. Future research may replace all of the above hypotheses but momentarily they serve as a convenient frame of reference. It is hoped that the interested reader will study critically forthcoming reports and add his own observations.

The authors wish to express their gratitude to Drs. W. R. McCrum and A. R. Kelly for their most helpful advice and criticism.

REFERENCES

1. Schopbach, R. R.: Review of psychopharmacologic drugs, Henry Ford Hosp. M. Bull. 7:290, 1959.

2. Hess, W. R.: Das Zwischenhirn; Syndrome, Lokalisationen, Funktionen, Basel, Schwabe, 1954.

3. Loewi, O.: Ferrier lecture on problems connected with principle of humoral transmission of nervous impulses, Proc. Roy. Soc., London (s.B) 118:299, 1935.

4. Marrazzi, A. S.: Effects of certain drugs on cerebral synapses, Ann. New York Acad. Sc. 66:496, 1957.

5. Vogt, M.: Concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs, J. Physiol. 123:451, 1954.

6. Gertner, S. B., Paasonen, M. K., and Giarman, N. J.: Studies concerning the presence of 5-hydroxytryptamine (serotonin) in the perfusate from the superior cervical ganglion, J. Pharm. & Exper. Ther. 127:268, 1958.

7. Grundfest, H.: General physiology and pharmacology of synapses and some implications for the mammalian central nervous system, J. Nerv. & Ment. Dis. 128:433, 1959.

8. Eccles, J. C.: Physiology of Nerve Cells, Baltimore, Johns Hopkins Press, 1957.

9. Palade, G. E., and Palay, S. L.: Electron microscope observations of interneuronal and neuromuscular synapses, Anat. Rec. 118:335, 1954.

10. Green, D. E.: Enzymes and the mode of action of sedative and hypnotic drugs, In, Miles-Ames Research Laboratory: Symposium on Sedative and Hypnotic Drugs, Baltimore, Williams & Wilkins, 1954, pp. 20-31.

11. Tepperman, J., and Tepperman, H.: Some effects of hormones on cells and cell constituents, Pharm. Rev. 12:301, 1960.

12. Green, D. E., and Lester, R. L.: Role of lipides in the mitochondrial electron transport system, Fed. Proc. 18:987, 1959.

13. Lehninger, A. L.: Enzymic and morphologic organization of the mitochondria, Pediatrics 26:466, 1960.

14. Baxter, C. F., and Roberts, E.: In, Symposium on the Neurochemistry of Nucleotides and Amino Acids, 1958. Ed. by R. O. Brady and D. B. Tower, New York, Wiley, 1960.

15. Tasaki, I.: Conduction of the nerve impulse, In, American Physiological Society: Handbook of Physiology, Washington, D. C., 1959, Sect. 1, v.1, p. 75.

16. Carlsson, A., Lindqvist, M., and Magnusson, T.: 3, 4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists, Nature 180:1200, 1957.

17. Everett, G. M., and Toman, J. E. P.: In, Society of Biological Psychiatry: Proceedings of the Scientific Sessions, 1958. Ed. by J. Masserman, New York, Grune & Stratton, 1959, Chap. 6.

18. Brodie, B. B., Olin, J. S., Kuntzman, R. G., and Shore, P. A.: Possible interrelationship between release of brain norepinephrine and serotonin by reserpine, Science 125:1293, 1957.

19. Brodie, B. B.: Rapid biosynthesis of brain serotonin before and after reserpine administration, Naturwissenschaften 10:243, 1958.

20. Plummer, A. J., Barrett, W. E., Maxwell, R. A., Finocchio, D., Lucas, R. A., and Earl, A. E.: Hypotensive properties of syrosingopine SU-3118, an ester of methyl reserpate, Arch. internat. pharm. dyn. 119:245, 1959.

21. Pletscher, A.: Release of 5-hydroxytryptamine by benzoquinolizine derivatives with sedative action, Science 126:507, 1957.

22. Brodie, B. B., Finger, K. F., Orlans, F. B., Quinn, G. P., and Sulser, F.: Evidence that tranquilizing action of reserpine is associated with change in brain serotonin and not in brain norepinephrine, J. Pharm. & Exper. Ther. 129:250, 1960.

23. Pletscher, A., Besendorf, H., and Gey, K. F.: Depresson of norepinephrine and 5-hydroxytryptamine in the brain by benzoquinolizine derivates, Science 129:844, 1959.

24. Mann, P. J. G., and Quastel, J. H.: Benzedine (β -Phenylisopropylamine and brain metabolism, Biochem. J. 34:414, 1940.

25. Anderson, E. G., and Bonnycastle, D. D.: Study of the central depressant action of pentobarbital, phenobarbital and diethyl ether in relationship to increases in brain 5-hydroxytryptamine, J. Pharm. & Exper. Ther. 130:138, 1960.

26. Oates, J. A., and Zaltzman, P.: Discussion of, Sjoerdsma, A., Gillespie, L., and Udenfriend, S.: Method for measurement of monoamine oxidase inhibition in man; application to studies on hypertension, Ann. New York Acad. Sc. 80:977, 1959.

27. Brodie, B. B., Spector, S., and Shore, P. A.: Interaction of monoamine oxidase inhibitors with physiological and biochemical mechanisms in brain, Ann. New York Acad. Sc. 80:609, 1959.

28. Loomer, H. P., Saunders, J. C., and Kline, N. S.: APA Psychiatric Research Reports No. 8, 1957.

29. Zbinden, G., Randall, L. O., and Moe, R. A.: Clinical and pharmacological consideartions on mode of action of monoamine oxidase inhibitors, Dis. Nerv. System 21: (Suppl.) 89, 1960.

30. Schopbach, R. R.: To be published.

31. Chessin, M., Kramer, E. R., and Scott, C. C.: Modifications of the pharmacology of reserpine and serotonin by iproniazid, J. Pharm. & Exper. Ther. 119:453, 1957.

32. Mullins, L. J.: Some physical mechanisms in narcosis, Chem. Rev. 54:289, 1954.

33. Ernsting, M. J. E., Kafoe, W. F., Nauta, W. Th., Oosterhuis, H. K., and Waart, C. de: Biochemical studies on psychotropic drugs. I. Effect of psychotropic drugs on γ-aminobutyric acid and glutamic acid in brain tissue, J. Neurochem. 5:121, 1960.

34. Porter, W. C.: In, Solomon, H. C., and Yakovlev, P. I., eds.: Manual of Military Neuropsychiatry, Philadelphia, Saunders, 1945, Topic 22.

35. Shurley, J. T.: Profound experimental sensory isolation, Am. J. Psychiat. 117:539, 1960.

36. Heath, R. G., Martens, S., Leach, B. E., Cohen, M., and Angel, C.: Effect on behavior in humans with the administration of taraxein, Am. J. Psychiat. 114:14, 1957.

37. Hoffer, A., and Osmond, H.: Adrenochrome model and schizophrenia, J. Nerv. & Ment. Dis. 128:18, 1959.

UNUSUAL SEIZURE DISCHARGES ON STIMULATION IN THE LIMBIC SYSTEM OF THE MONKEY*

L. D. Proctor, M.D.,** W. R. McCrum, Ph.D.,** J. Lukaszewski, M. A.**

INTRODUCTION: During the course of an investigation concerning the effects of stimulation of the reticular formation and limbic system upon behavior in the monkey (Macacca Mulatta), we have observed some electrographic seizure activity in the amygdala which was, in our experience, unique.

After establishing the fact that we could consistently reproduce this activity in the temporal lobe of either hemisphere of our monkey, we consulted with Dr. Gloor[1] who conceded that the discharges were indeed somewhat unique; but he suggested further experience with other monkeys before such activity could be considered to have much significance.

We have at this time completed a similar set of experiments in another monkey and have been able to duplicate our results.

The method of procedure, both for stimulation and recording in the limbic system includes the implanatation of a Jasper-Delgado type of electrode with four leads, having a distance of 2.5 mm. between adjacent pairs of contact tips.[2,3] These are placed stereotactically in the temporal lobe with the deepest contact in the amygdaloid nuclear complex. The leads are connected to the input of Techtronix 502 low level pre-amplifiers and the output of these is fed simultaneously to an Offner transistor electroencephalograph, an Ampex 1103 tape recorder and an Offner frequency analyzer.

Silver—Silver chloride scalp leads are attached with collodion and connected directly to the Offner Electroencephalograph.

At the conclusion of the experiments, the animals are sacrified, their brains profused and the electrode placement grossly verified. The specimen is then prepared for microscopic examination.

With previous experience we had established a stimulus pattern consisting of square wave pulses from a Grass SB-4 stimulator delivered at 400 cycles per second with a duty cycles of 0.8%. The voltage setting was often varied but always monitored as also was the current by an oscilloscope.

Such a stimulus pattern often produced motor responses and electrical after-discharges, but prolonged electrographic seizures were never observed. During a series of stimulation with different parameters in an attempt to bracket motor response thresholds, the duty cycle of the square wave was increased from 0.8% to 80%. When this was done prolonged electrographic seizure activity was invariably observed with the proper voltage setting.

OBSERVATIONS: The short duration electrical discharges (or after-discharges), which appeared immediately following cessation of stimulation (Fig. 1, 2) were

*This investigation was done under N.I.H. grant M 2474.
**Department of Neurology and Psychiatry. Presented at the meeting of the Eastern Association of Electroencephalographers, New York City, December, 1960.

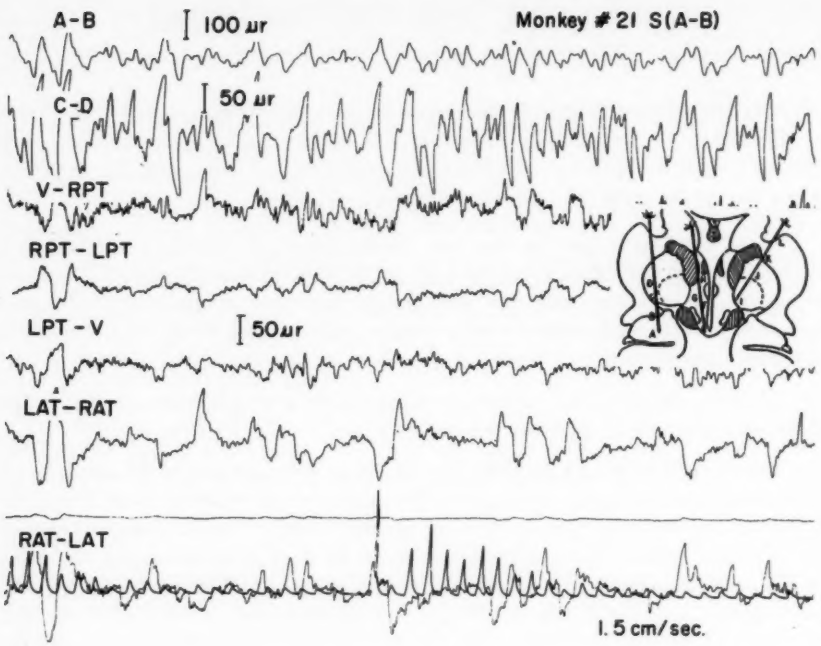


Figure 1
After-discharge immediately following stimulation of the amygdala. (A-B) and (C-D).

characteristic in form and frequency with previously reported amygdaloid seizures.[4,5] They lasted from a few seconds to a few minutes and neither their appearance nor duration appeared related to the magnitude of the duty cycle. As in other reports,[6,7] the cerebral cortex seemed remarkably free from this amygdaloid activity; however, other deep brain structures, forebrain nuclei, ganglia and hypothalamus frequently showed involvement. (Fig. 2).

The seizure discharges, which seemed to us unique, were always delayed in onset after cessation of the stimulus. This delay varied from one to more than ten minutes. If the stimulus produced an immediate after-discharge, then the onset of the prolonged discharge was delayed even further. The onset of this delayed seizure was usually heralded by a shift in the baseline (Fig. 3) equivalent to as much as 1200 microvolts in amplitude with a subsequent dip below the baseline and return to resting level. The entire biphasic process lasted about two to three seconds. The paroxysmal activity would immediately follow. The initial amplitude was usually low but built up slowly to as high as 200 microvolts. The dominant frequency was usually about 2½ to 3 cycles per second, although this (Fig. 4) frequency would shift to harmonics of 6, 12, and 24 cycles per second. The duration of these discharges was in some instances only four or five minutes; however, on two occasions the seizure lasted for over an hour and one seizure continued uninterrupted for an hour and fifty minutes.

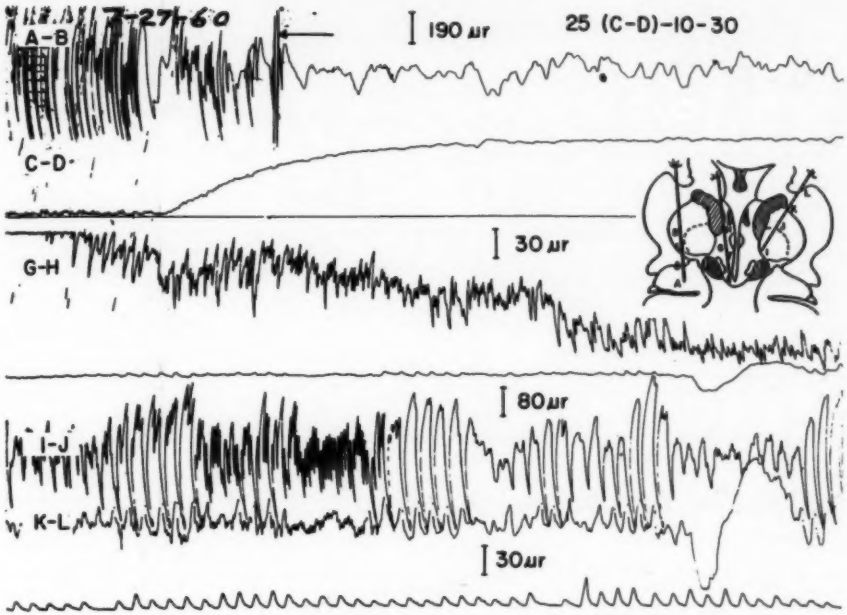


Figure 2

After-discharge spreading to hypothalamus (G-H) and contralateral basal ganglia (I-J) and (K-L).

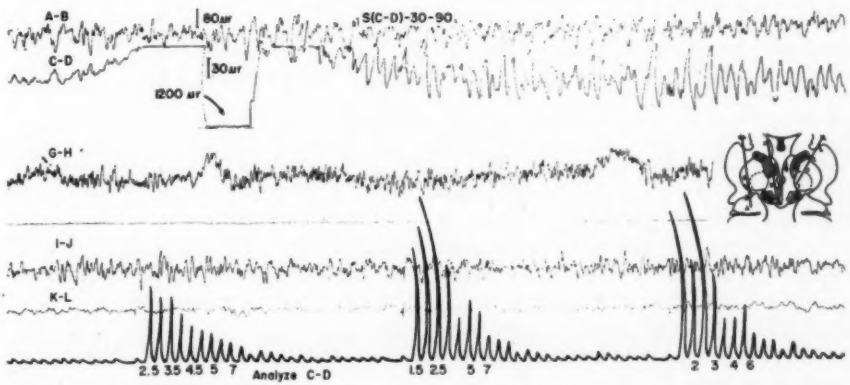


Figure 3

Delayed seizure discharge preceded by high amplitude "biphasic shift" of the baseline. (C-D).

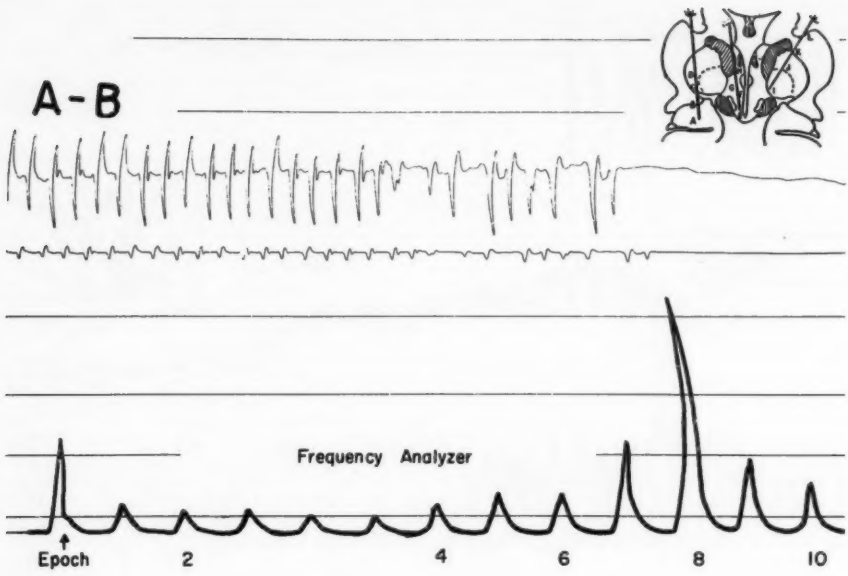


Figure 4

Shifting of frequency spectrum and "harmonics".

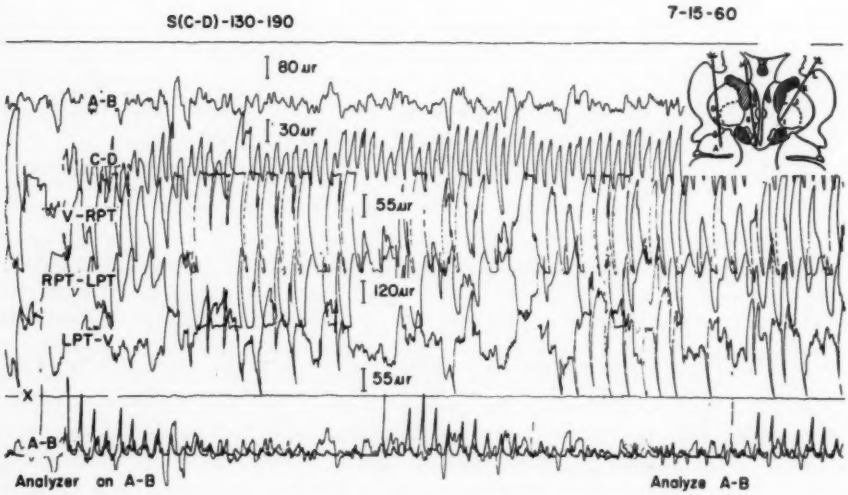


Figure 5

A rare cortical response during a delayed seizure. (V-RPT) (RPT-LPT) and (LPT-V)

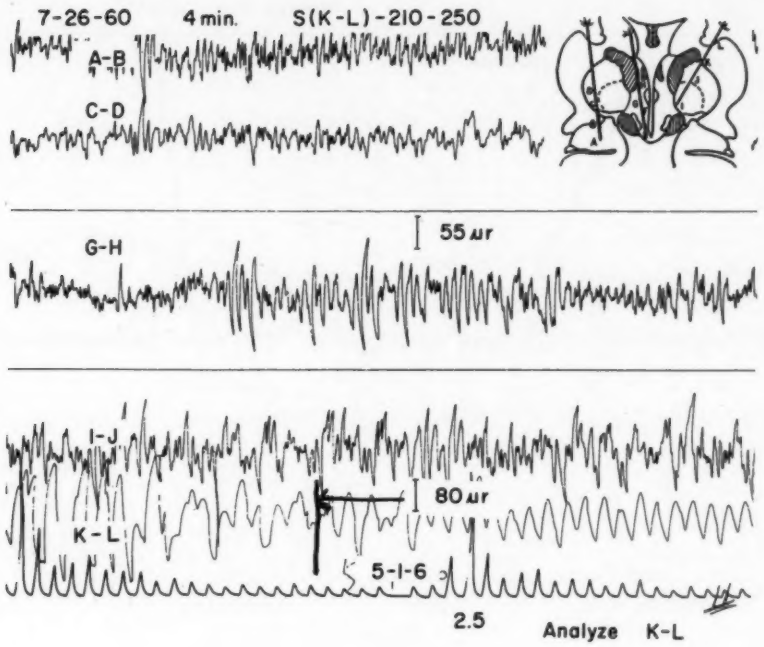


Figure 6

Hypothalamic response (G-H) during a delayed seizure.

These discharges were similar in their anatomical distribution to the after-discharges. On rare occasions they were seen in the cortex (Fig. 5) and hypothalamus (Fig. 6) and were frequently seen in the amygdala and in the basal ganglia of the opposite hemisphere (Fig. 7). Activity in these areas was delayed in appearance until the activity in the original site was well established, and it was seldom maintained for more than a few minutes.

Delayed, prolonged seizures were produced in both hemispheres with simultaneous stimulation of the amygdala of both hemispheres, but the time of onset of the discharge between the two sides was always different.

Behavior concomitants of unilateral limbic system stimulation have been described in a previous publication.[8] With bilateral stimulation of this system, in addition to the head turning, mouth and tongue movements, etc. noted previously,[8] a snake-like darting of th tongue was seen.* Minimal changes in states of consciousness were suspected as the animal appeared to stare from time to time during the prolonged electrical discharges. Automatic repetitive movements of the hand also occurred. Reliable psychometric measurements were not possible on the two monkeys used in this investigation.

*A movie film was shown of this behavior.

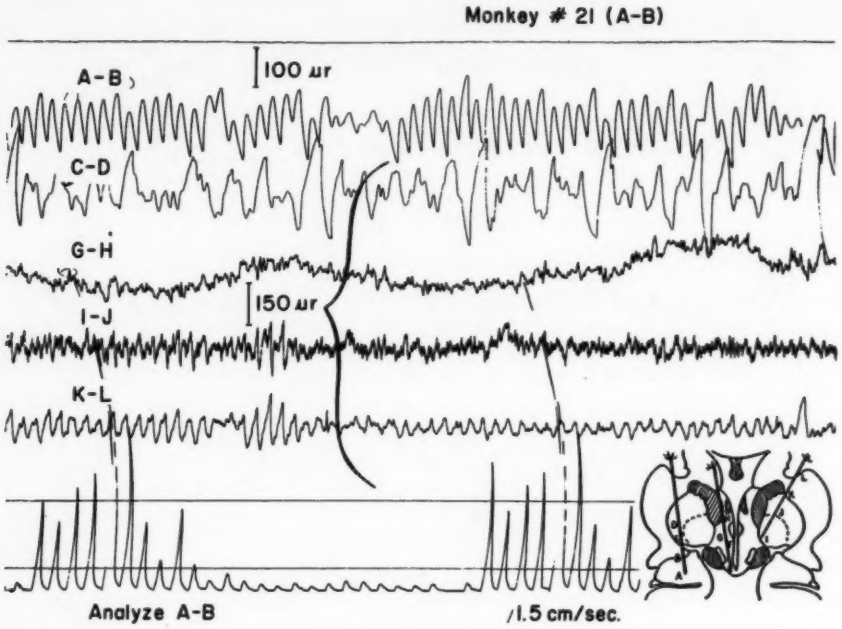


Figure 7

Spread of a delayed seizure (A-B) to the deep structures of the contralateral temporal lobe (I-J) and (K-L).

An anatomical examination both grossly and histologically showed that in one monkey the left electrode had its deepest contact in the core of the amygdaloid complex and its upper most contact near the anterior commissure. The right electrode had all four contacts within the globus pallidus and putamen (Fig. 8). In the other monkey both electrode tips were within the amygdaloid complex but the left one was a few millimeters deeper. One monkey had an electrode in the diencephalon with the deepest contact in the left ventromedial nucleus of the hypothalamus.

DISCUSSION: As can be seen from the evidence, we are dealing with a complex phenomenon which implies an interaction of several, at least, partly independent systems or circuits. The rhythmic discharge is localized in its appearance yet may be propagated to distant structures; however, the delay in appearance at the stimulus site and the delay in its propagation to other structures suggest an intrinsic genesis of the wave form rather than an extrinsic driving mechanism. Understanding of the nature of the mechanisms involved is certainly beyond the scope of the present findings. Further studies of micro-electric correlates and the vectors of stimulus parameters should be of help in this regard.

The above findings have shown a quality of temporal lobe electrical activity which is consistent both physiologically and anatomically with repeated experience. It is hoped that further study of this phenomenon will add to the concept of temporal lobe seizure activity.

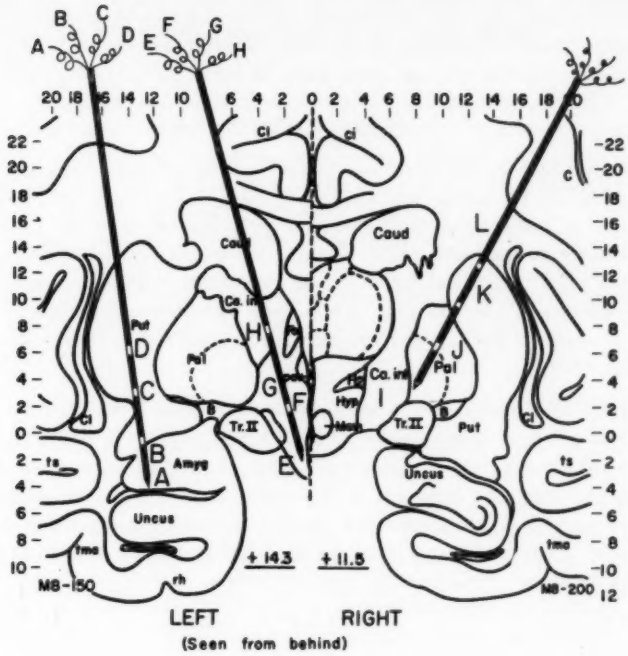


Figure 8

Placement of electrodes in monkey No. 21.

The authors wish to acknowledge the assistance of H. van den Ende, E. E. and H. F. Labsan, M. D. in this work.

REFERENCES

1. Gloor, P.: (personal communication).

2. Proctor, L. D., Knighton, R. S., and Churchill, J. A.: Variations in consciousness produced by stimulating the reticular formation of the monkey, Neurology 7:193, 1957.

3. Van den Ende, H.: A method for stimulation and simultaneous electroencephalogram recording, Henry Ford Hosp. M. Bull. 7:174, 1959.

4. Arana-Iniguez, R., Reis, D. J., Naquet, R., and Magoun, H. W.: Propagation of amygdaloid seizures, Acta Neurol. Latinoam., 1:109, 1955.

5. Kaada, B. R.: Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of 'Rhinencephalic' and other structures in primates, cat and dog, Acta Physiol. Scand. 24:1, Suppl. 83, 1951.

6. Feindel, W., and Gloor, P.: Comparison of electrographic effects of stimulation of the amygdala and brain stem reticular formation in cats, Electroencephalog. & Clin. Neurophysiol. 6:389, 1954.

7. Tokizane, T., and Sawyer, C. H.: Sites of origin of hypoglycemic seizures in the rabbit, A.M.A. Arch. Neur. Psychiat. 77:259, 1957.

8. Proctor, L. D., Knighton, R. S., Lukasewski, J., and Bebin, J.: Behavioral changes during hypothalamic or limbic stimulation in the monkey, Am. J. Psychiat. 117:511, 1960.

PYOGENIC OSTEOMYELITIS: THE INFLUENCE OF BONE REMODELLING ON RECURRENCE

H. M. Frost, M.D.*

INTRODUCTION

It is known that streptococcal and pneumococcal pyogenic osteomyelitis exhibit a strong predilection for infants and that recurrences of these two entities in infants is uncommon. It is known that staphylococcal pyogenic osteomyelitis exhibits a predilection for older children and adults, and that recurrences are more likely than when the etiological agent is a streptococcus or pneumococcus. Recurrences are more likely when the disease occurs in the adult. Recurrence in osteomyelitis affecting cancellous bone, such as vertebral body, is less common than recurrence in osteomyelitis affecting cortical bone.[1,2,3,4,20,21]

Previous publications have pointed out that the normal bone spaces,** numbering over 1,000,000 per cubic millimeter of cortex,[10] act as a bacterial reservoir in pyogenic osteomyelitis. Microorganisms invading bone infest all of these spaces until their source of nutrients-dead cells and tissue — is exhausted. At this stage the streptococci and pneumococci seem to die off, explaining the low recurrence rate and the more benign aspect of these infections as compared to staphylococcal ones. Staphylococci manage to survive in the bone spaces in some manner, perhaps as protoplasts, and can remain potentially infective for observed periods as long as 70 years. This *bacterial reservoir of pyogenic osteomyelitis*[7] makes staphyloccal osteomyelitis and perhaps osteomyelitis due to a few other bacterial agents, peculiar.

Diffusion impedence is the resistance to diffusion from one point to another experienced by molecules in a solution.*** A high diffusion impedence normally occurs in fresh bone in vitro. A high diffusion impedence has been observed in bone with the tetracycline antibiotics in man in vivo. It is probable that this diffusion impedence affects other antibiotics in bone also.[11,15] Diffusion impedence is particularly high in the case of bone that is dead in vivo.[5,6] It is probable that the prolonged antibiotic dosage empirically found necessary in treatment of pyogenic osteomyelitis is due primarily to the high diffusion impedence affecting the antibiotics in bone. It is thus likely that with short dosage times the spaces of the bacterial reservoir of pyogenic osteomyelitis do not develop sufficient concentrations of the antibiotic to sterilize them.

In this paper the effect of remodelling on the bacterial reservoir will be pointed out. By remodelling is meant the sum total of bone formative and destructive processes. These processes may be considered in two parts. The first comprises the internal remodelling which goes on for the duration of life and which is gradually responsible for the removal of old bone and replacement by new bone. The second comprises the more active, and morphologically more distinctive, processes associated with growth.

It will help in the following discussion if the reader will visualize the portion of the bone involved in osteomyelitis as being distinctively labelled. In actuality this

*Department of Orthopaedic Surgery.

**These spaces are the marrow spaces, vascular channels, osteocyte lacunae and canaliculae.

***The solution may be gaseous or solid as well as liquid.

part of the bone is distinguished by greater numbers of dead osteocytes and dead vascular channels, and by the presence of microorganisms in these spaces. In an analogous sense this dead, bacteria-containing bone may be thought of as labelled a bright yellow. The effect of remodelling processes on this yellow, labelled bone may then be visualized as a removal of it and replacement of it with unpainted, uncontaminated, live, normal bone. There is more to this analogy than mere speculation. The recently discovered labelling of bone with tetracyclines produces such an effect in vivo which is permanent until remodelling processes remove the labelled bone, and which can be observed and measured for observed periods as long as 9 years between labelling with tetracycline and examination of the skeleton at post-mortem.[8,15,16,17,18,19] It is with the aid of tetracycline labelled human skeletons that the essential information given in the following paragraphs was obtained.‡

REMODELLING

Internal Remodelling and the Bacterial Reservoir of Pyogenic Osteomyelitis

For many years it has been realized that a ceaseless destruction of existing bone, and replacement with new bone, goes on in the skeleton regardless of the age of the person. The rate at which this internal remodelling and reconstruction occurs has been a matter of guesswork and of analogy from animal to man. Prior to recent methods and work the normal, internal remodelling could not be measured in man.

It has been learned that normal, internal remodelling is characterized by the elaboration of osteoid seams during the formation of lamellar bone,[9,13] that lamellar bone is the normal adult skeletal constituent, and that the formation of osteoid seams and the formation of tetracycline labelled bone bear a reasonably constant relationship to each other.[8] Armed with this information, and additional data which need not be elaborated here, it has been possible to measure remodelling activity in man in terms of new bone formation.[12] A representative curve of remodelling activity in human rib by age is given in Figure 1. At two points on the curve a quantitative measurement is designated in terms of the percentage of the absolute bone volume‡‡ that is torn down and remade per month. It will be seen that internal remodelling is very active at one year of age but slows down markedly during adulthood, being lowest about age 30. If we express remodelling rates in terms of the biological half-life‡‡‡ of the bones, the figures are more meaningful. At age one year the half-life is about .25 year, while at age 30 it is 15 years and at age 60 about 8 years. At age one, a year of normal remodelling will remove over 93% of the contaminated bone that is called the bacterial reservoir, and replace it with normal, uncontaminated bone. At age 30 a year of normal remodelling will remove less than 5% of the bacterial reservoir.

It can be seen that the active remodelling processes of the very young will remove nearly all of the bacterial reservoir, given sufficient time; that this removal will be

‡It should be understood that in many instances tetracycline labelled material has merely confirmed estimates made by other means, or analogies made from animal investigation.

‡‡Absolute bone volume: the volume of bone remaining after subtraction of the volumes of the osteocyte lacunae and canaliculae (about 2%), the vascular channels (5-10%) and the marrow spaces.

‡‡‡The time required for remodelling processes to reconstitute half the absolute bone volume if remodelling continued (at the measured rate) indefinitely.

less complete the older the child at age of onset of his disease; and that the contaminated bone will never be completely removed by this means. A moment's reflection and an analogy will reveal why. There was once, in Greek mythology, a hare who ran a race and who covered first half of the distance, then half the remaining distance, then half that remainder and so on to infinity, so that in the analogy he never made the finish line. In the present case, the analogy happens to be true rather than fallacious. During a normal life there would always be some contaminated bone remaining, unremodelled.

In the above discussion several factors have been omitted but must now be mentioned for the sake of completeness. First, there is great individual variation in remodelling rates among different patients.[9,12,14] Second, a thoroughly sequestered piece of cortex does not undergo much internal remodelling, although in time it may be completely destroyed or evacuated.[1,21] Third, in the presence of adjacent infection the remodelling rate in a bone increases so that the values given in Figure 1

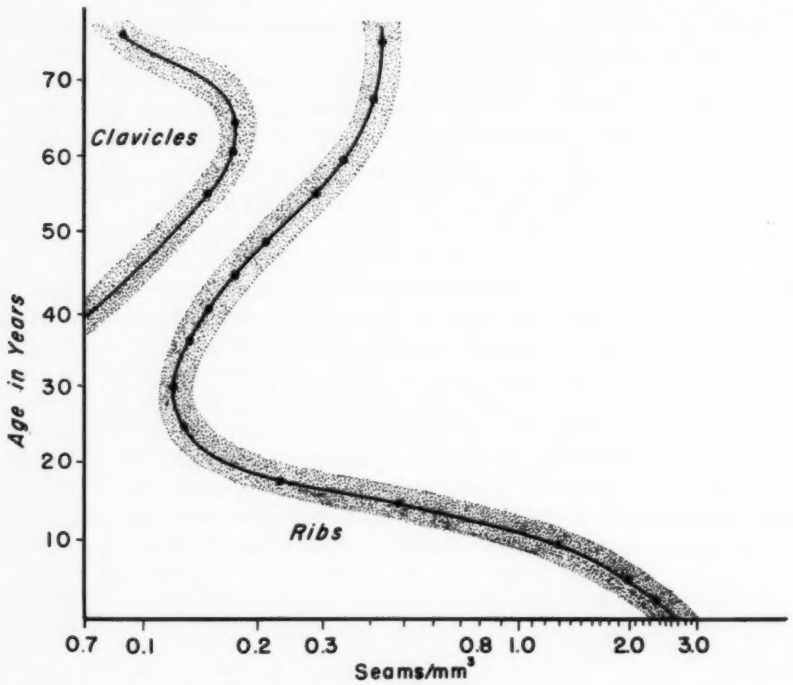


Figure 1

Osteoblastic activity in ribs and clavicles plotted against age. The activity was measured as seams/mm³ of cortex in 40 clavicles and 65 ribs. While the "Y" axis is a linear plot, the "X" axis is logarithmic. There is a rapid decrease in internal remodelling activity in the first 20 years of life. By planimetric methods applied to human, tetracycline labelled bone, it is known that the bottom dot on the rib scale corresponds to a biological half-life of 0.25 year, while the 5th dot from the top corresponds to a biological half-life of 8 years.

(Reproduced by permission Henry Ford Hospital Medical Bulletin, 9, 1961)

are low for what would be expected in the case of pyogenic osteomyelitis. A case in point is the fibula of a 47 year old man. There was an osteomyelitis of the tibia. The half life of the femur in this extremity was 47 years while that of the fibula was 2.3 years, the latter being a very high rate for a bone of the appendicular skeleton in an adult.

Growth Remodelling and the Bacterial Reservoir of Pyogenic Osteomyelitis

The second type of remodelling is that due to growth and so is peculiar to children. The effect this will have on the amount of bacterial reservoir remaining after an attack of osteomyelitis, and the effect of the age of the child at the time of onset of the disease, is illustrated in Figure 2. A representative bone, the tibia, is viewed in both cross and longitudinal section at age one, two and 7 years. The original, contaminated bone is shaded. It is seen that as the diameter and length of the bone

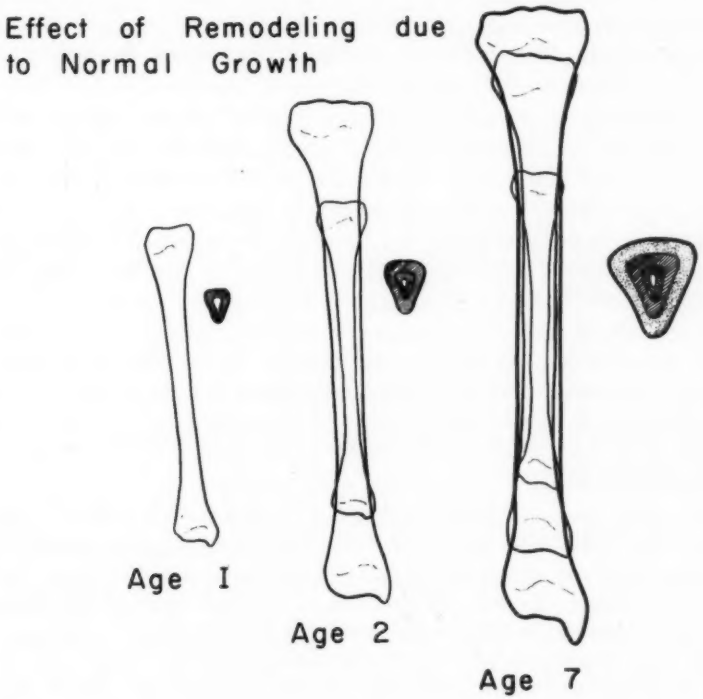


Figure 2

Diagrammatic presentation of the effect of growth on the composition of older bone — the left diagram is that of a tibia of a child one year old, seen from the front and in cross section. On the right the effect of 6 years of growth is seen in similar views. The cortex of the one year old has been completely removed by age 7. Accordingly any bacteria residing in osteomyelitic bone at age one would be removed entirely by age 7 and so could not cause a recurrence of osteomyelitis.

enlarge due to growth, the original bone is progressively removed. All of the bacterial reservoir would be removed provided the child were young enough at the time of onset of the disease, or provided the amount of contaminated bone were sharply limited by vigorous early treatment of the disease, by low virulence of the organism, by high resistance to the infection and by other factors.[21] In some cases the disease might be so advanced before it is arrested that a degree and type of damage would result which prevents subsequent growth remodelling from removing all of the bacterial reservoir. It can be appreciated that in the older child it is less likely that growth remodelling will remove all the bacterial reservoir, and that near skeletal maturity this is impossible.

DISCUSSION

The course of any single case of pyogenic osteomyelitis is the result of interplay of so many different factors that it will be very difficult to recognize, evaluate and assign quantitative importance to all of them. In this paper and its predecessors[7,15] an attempt is made to discuss and bring to the clinician's attention some pertinent newer information. The significance of this information on the pathogenesis and course of the disease must be discussed.

The basic source of bacteria for recurrence of pyogenic osteomyelitis is the bacterial reservoir present in the physiologic spaces of contaminated bone following an attack of pyogenic osteomyelitis. In the case of bacteria such as streptococci and pneumococci, exhaustion of the culture medium apparently brings on the death of the organisms, because recurrences are rare when these organisms are the etiological agent. Staphylococci are different, being able to survive in some manner for many years in contaminated bone.

As a result of these peculiarities three generically different types of phenomena become of interest when dealing with the residuals of a staphylococcal osteomyelitis. The first is the sensitivity of the organism in its peculiar dormant state to antibiotics. This sensitivity may be quite different from the sensitivity of the actively metabolizing organisms in an active infection. This matter is better considered by the bacteriologist than the pathologist.

The second genus of phenomena may be summed up in the term "diffusion impedence." The antibiotic molecules do not permeate bone as readily as they permeate soft tissues, and in particular they do not permeate dead bone very well. This makes it difficult to obtain adequate concentrations of antibiotic agents in the multitude of spaces in contaminated bone that contain viable organisms. It is easy to visualize a nest of staphylococci, in dormant but viable form, suddenly activated years after the initial attack of osteomyelitis when remodelling processes remove the wall of their bony sarcophagus and thus expose them to the normal body fluids with their contained nutrients. Sterilization of the spaces in the bacterial reservoir becomes a matter of prolonged treatment with antibiotics. In the future it may also become a matter for antibiotics whose physicochemical properties are tailored to the peculiarities of the task.

The third genus of phenomena has already been discussed, and consists of the gradual removal of the bacterial reservoir by internal and growth remodelling. The

latter is the more effective process. This explains the increasing frequency of recurrence of staphylococcal osteomyelitis with increasing age of the patient at the time of onset.

SUMMARY

The existence of a bacterial reservoir inhabiting the normal spaces in bone after an attack of pyogenic osteomyelitis has been noted. The peculiar ability of staphylococci to remain in these spaces in a potentially infective state for many years has also been noted.

The internal remodelling of bone that occurs all during life gradually removes this bacterial reservoir and replaces it with healthy, uncontaminated bone. This process is much more active in children than in adults. It has recently become possible to measure it quantitatively. The remodelling rate may be expressed as the number of years required to reconstitute half of the absolute bone volume. In these terms, normal biological bone half-lives are, at age one: 0.25 years, age 30: 15 years, age 60: 8 years. While internal remodelling is relatively effective in removing the bacterial reservoir in children, it is relatively ineffective in adults but could not completely remove the bacterial reservoir in either. There normally is some acceleration of internal remodelling in bone adjacent to dead, osteomyelitc bone so that the figures given are in reality low in the presence of the disease being discussed. In sequestered bone, remodelling is nil for practical purposes. A sequestrum must be destroyed or evacuated.

Growth remodelling is capable of completely removing the bacterial reservoir. This is more apt to occur when the onset of the disease is early, when treatment is early and vigorous, when other factors act to minimize the amount of bone contaminated, and when the disease does not markedly interfere with subsequent growth remodelling.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopaedic Diseases, Philadelphia, Saunders, 1958.

2. Altemeier, W. A., and Wadsworth, C. L.: Evaluation of penicillin therapy in acute hematogenous osteomyelitis, J. Bone & Joint Surg. 30A:657, 1948.

3. Beerman, C. A.: Treatment of acute hematogenous osteomyelitis of the long bones in infants and children, J. Pediat. 33:578, 1948.

4. Casagrande, P. A., and Frost, H. M.: Fundamentals of Clinical Orthopaedics, New York, Grune & Stratton, 1953.

5. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

6. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

7. Frost, H. M.: Pyogenic osteomyelitis: Normal bone spaces as a bacterial reservoir, Henry Ford Hosp. M. Bull. 8:263, 1960.

8. Frost, H. M.: Human osteoblastic activity. II Measurement of biological half life with some results, Henry Ford Hospital M. Bull. 9:87, 1961.

9. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

10. Frost, H. M.: Measurement of osteocytes per unit volume and volume components of osteocytes and canaliculae in man, Henry Ford Hosp. M. Bull. 8:208, 1960.

11. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

12. Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurements with some results, Henry Ford Hosp. M. Bull. 9:76, 1961.

13. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

14. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. 35:179, 1960.

15. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:255, 1960.

16. Frost, H. M., Villanueva, A. R., and Roth, H.: Tetracycline staining of newly forming bone and mineralizing cartilage in vivo, Stain Technol. 35:135, 1960.

17. Frost, H. M., Villanueva, A. R., and Roth, H.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

18. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

19. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

20. Trueta, J.: The three types of acute haematogenous osteomyelitis; Clinical and vascular study, J. Bone & Joint Surg. 41B:671, 1959.

21. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.

VISUAL AIDS AND THE MEDICAL STAFF

WILLIAM E. LOECHEL*

Perhaps the two most frequently asked questions relating to the portrayal of medical subject matter are:

1. "Hasn't photography replaced drawing?", and
2. "How long does it take to produce one of these drawings?"

The answer to the first question is simply "No . . . they are allied fields that work for and with each other". This brief article will attempt to answer the second question.

The time that is taken in producing medical drawings, charts, exhibits or models is based upon several factors; i.e., clear idea of the requester, availability of other services if involved, and complexity of the subject. With these thoughts in mind, the following suggestions are set forth, with the idea of giving the staff the best service from the Medical Illustration Department.

SURGICAL DRAWINGS:

If it is possible to alert the Department of Illustration before an operation is scheduled, it is wise to do so. Once the illustrator is present, it will be up to the surgeon to place him in a position of seeing the most for the time he spends there. While it is not necessary to keep up a running conversation, the operator should keep in mind points to be emphasized in the drawing, possibly alerting the illustrator before that particular stage is reached.

Should it happen that an illustrator is unavailable, the surgeon can still have the drawings made later after sufficient consultation. Photos taken of operative fields are valuable to the illustrator in many cases, but the most efficient way to have surgical drawings made is to have the illustrator at the table.

GROSS SPECIMENS:

Many gross specimens that do not lend themselves readily to photography, find their way to the laboratory shelves without any thought being given as to whether or not they can be diagrammed or drawn. If such a specimen is of sufficient interest to the doctor, it would be well worth his while to have drawings made of it during a relatively fresh state before it has a chance to become distorted. As an example, a heart specimen was selected from the laboratory shelves which had not previously been photographed or drawn. Its significant features were atrial septal defect, ventricular septal defect, hypertophied right ventricle, and a hypoplastic left ventricle. Drawings of the specimen are shown. One should note also the anomalous positions of aorta and pulmonary vein. These drawings were made with the idea of reconstructing the subject as it appeared in the living subject with the added visualization of cut away areas and cross sections. While the left half of the illustration was made by simply approximating the cut edges of the postmortem incision, the right half was produced by schematizing information gotten from many views of all the chambers. The drawing provides a clear visual picture and no tissue was destroyed in producing it. (Fig. 1).

*Director, Medical Illustration Department.

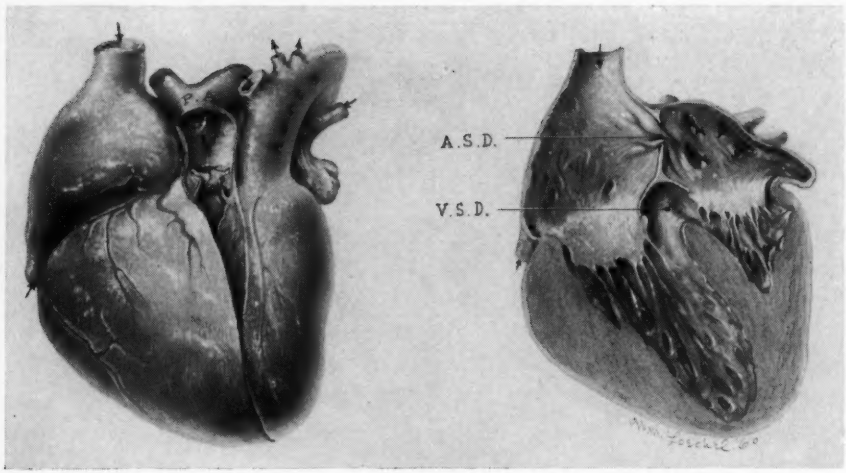


Figure 1

During a postmortem examination, some anatomical features are better shown by a drawing than a photograph.

During a postmortem examination, some anatomical features worth recording are better shown by a drawing than by a photograph. On one occasion, a call from the autopsy room came at 10:30 P.M. The doctor had found extremely unusual relationships in the chest and felt that to draw the structure in situ would be a valuable document. The body had to be ready for the mortitian to take early in the morning. Drawings were made showing the heart, lungs, anomalous vessels, rib cage, etc., as they appeared; in addition, a "phantom" drawing was made showing the hidden relationships. Time and service were put to good use.

In preparing to dissect structures related to porta-caval anastomosis, the author was confronted with the unusual relationships depicted in the accompanying drawing. An original pencil sketch took 10 minutes and the inking took ½ hour. (Fig. 2). This type of "comprehensive" drawing is a valuable record because it takes less time to understand than a written description.

Any number of interesting and valuable anatomical facts can be readily (and rapidly) recorded over a period of years. The writer encourages use of drawings in this area, and would be delighted to demonstrate simple drawing techniques that the doctors can use themselves in the course of their autopsy examinations.

EXHIBITS:

Assuming an exhibit plan has been approved, the requester should assemble as much of his material as possible and discuss this with the Medical Illustration Department. A six month advance notice is required. Once the basic discussion has taken

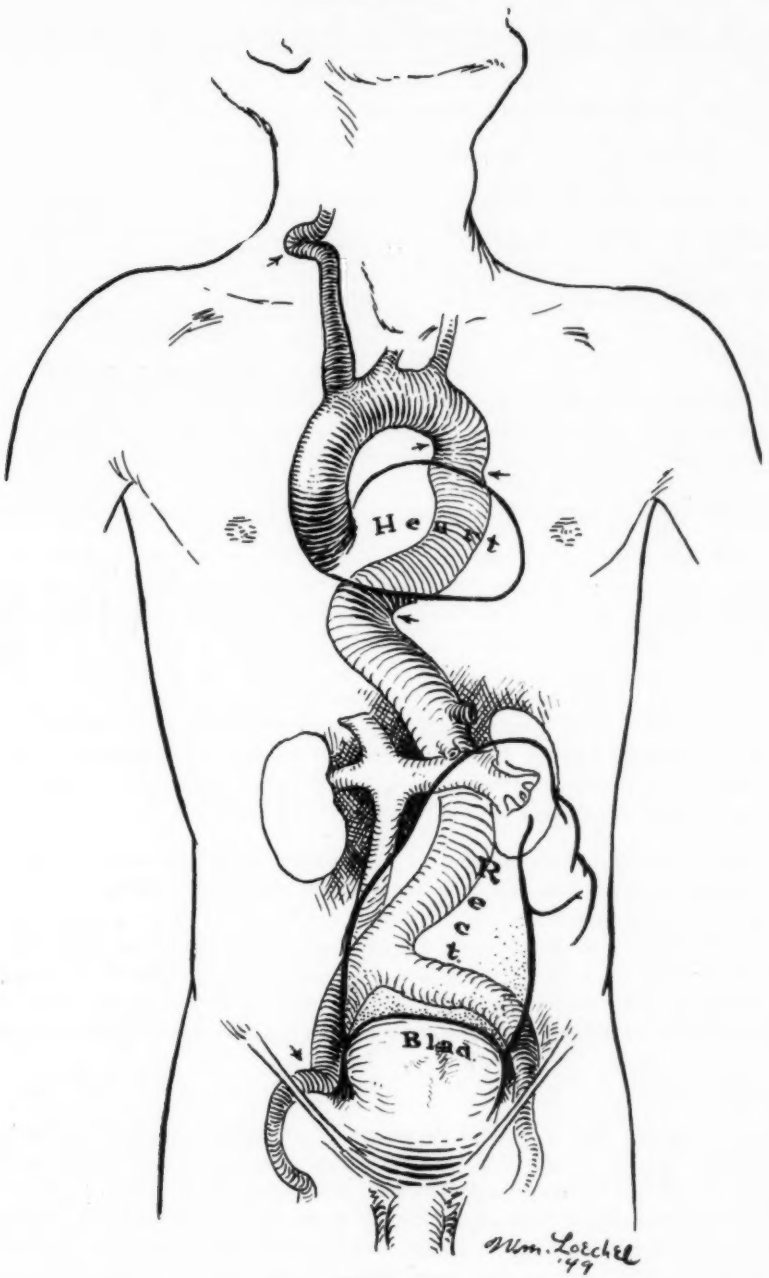


Figure 2
Drawing of in situ relationships employing "phatom" effect. (from one of the author's cadavers)

place, the material can be developed in an organized fahion; the usual progression being:

1. An overall design of the panels.
2. Mechanical or electrical details.
3. Specific illustrative details.

Crowding an exhibit with copy is to be avoided. In many cases, even the non-mechanized and sparsely labelled exhibit becomes animated because the physician in attendance can talk about the display.

MODELS:

Medical models can be produced only if sufficient time is allowed. Those contemplating such a project should consider thoroughly what is to be demonstrated, and the model built only after basic drawings (scale plans) have been agreed upon.

CHARTS AND GRAPHS:

Blue line graph paper is the ideal paper on which to develop a graph, and the requester who uses it makes the job simple for himself. The blue lines will not reproduce. If graph lines are to be included, black or red graph paper should be used. Drawn lines should be drawn with a pencil hard enough to retain its point, should there be converging lines. If it is possible to type the copy, this is preferable to writing. So often, terms in one field are clear to those in that field, even though hurriedly written, but when they appear before one who knows little of their meaning, the result is a stumbling block in the production of the otherwise clear preliminary drawing. To letter in or draw in anything that is not positively clear, often results in last minute patching and erasing.

Should the occasion arise when a great number of graphs are to be prepared, the machinery in the Illustration Department will run more effectively if the work is sent in early enough so that the preparation can be spread over a period of several weeks. To submit such an order on one day and expect it completed within three or four days would be unfair to others who are waiting for their jobs to be completed. If graphs are being developed by the doctor over a period of months, it is better to submit them as individual items than to wait until a collection of them is complete.

SLIDES:

As a general rule, if a sheet of ordinary typing paper is folded into quarters, any typing that can be put into the space (even crowding it) will be the optimum copy to be lettered on the slide. In this way, *all* lettering will be visible in an average auditorium. (Fig. 3). If need be, quite a bit more copy can be included, but its size will mean it is less legible from distant points. The important thing to remember is proportion. Where extensive copy must appear on the chart, emphasizing by means of color helps break up the mass of wording. Slides may be prepared with an overall tint, which takes little time to add and which makes the slides much more readable.

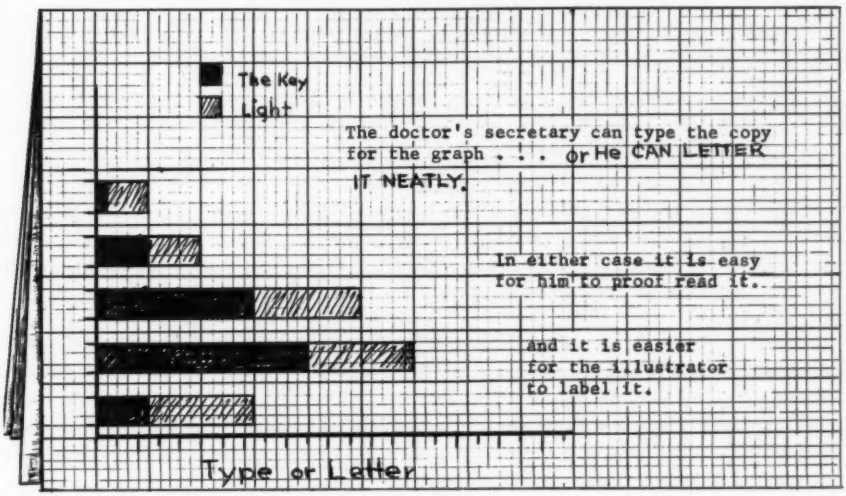

The Key
Light
The doctor's secretary can type the copy for the graph . . . or He CAN LETTER IT NEATLY.
In either case it is easy for him to proof read it.
And it is easier for the illustrator to label it.
Type or Letter

Figure 3

The Medical Illustration Department is eager to discuss the needs of the staff — feeling quite strongly that well planned visual descriptions are a vital part of a medical message.

X-RAY DIFFRACTION POWDER DATA FOR STEROIDS

JONATHAN PARSONS, WILLIAM T. BEHER, AND GIZELLA D. BAKER*

The systematic collection of x-ray powder diffraction data for steroids began in this laboratory early in 1955 and has been published at intervals in Analytical Chemistry.[1,2,3,4] In 1958 a compilation of all data obtained including pattern photographs and an index table following the indexing system of the X-ray Powder Data File[5] was published in the Henry Ford Hospital Medical Bulletin.[6] The present data includes sets of interplanar spacings, relative intensity determinations, and pattern photographs for 27 additional steroids. This set brings the total of powder steroid patterns, obtained by the authors, to 166.

The x-ray source equipment used for this work was a Norelco x-ray diffraction unit fitted with a copper tube and a nickel filter for isolating the Kα line. Norelco 114.59 mm. Debye-Scherrer cameras were used. The 200-mesh powdered specimens were mounted in thin-walled glass capillaries as previously described.[5] The usual procedure was to operate the diffraction tube at 35 KV potential and 20 ma. tube current for 2 hours. Films were developed for 5 minutes in Kodak X-ray Developer at 68° F.

Table I is a classified index of the steroids studied, including their respective melting point ranges. Established nomenclature has been used to characterize each compound. In order that one may quickly note the respective number of carbon, hydrogen, and oxygen atoms in the compounds, the molecular formulas are also included. Since many of these compounds exist in two or more polymorphic forms, this fact must be kept in mind when using these data for identification. When possible, samples should be recrystallized using the specific solvent indicated. In this study, all steroids were recrystallized from ethanol unless otherwise indicated in Table I. Melting points, determined on a Fisher-Johns melting point apparatus, are uncorrected.

The numbers on the photographs in Figure 1 and the column headings of Table II both correspond to the pattern numbers of the compounds given in Table I, which thus serves as a key to both the photographs and the x-ray diffraction data. Table II lists the interplanar spacing and relative intensity data for each steroid. The relative intensities were determind by visual comparison, using a calibrated set of intensity strips. The photographs of patterns (Figure 1) are positive prints made from a negative obtained by photographing the diffraction pattern films upon an illuminating table.

ACKNOWLEDGMENTS

The steroids used for this study were obtained from the Chemotherapy National Service Center, National Cancer Institute, U. S. Public Health Service, Bethesda, Maryland, with the kind assistance of Mr. Morris Graff. The authors are grateful to Dr. Michael J. Brennan, Henry Ford Hospital, for his encouragement and interest in the application of these data to clinical problems. The technical assistance of Mrs. Dorothy Huebner is also acknowledged with thanks.

*The Edsel B. Ford Institute for Medical Research, Department of Physics.

LITERATURE CITED

1. Parsons, J., and Beher, W. T.: X-ray diffraction powder data for steroids, Anal. Chem. 27:514, 1955.
2. Beher, W. T., Parsons, J., and Baker, G. D.: X-ray diffraction powder data for the steroids, Anal. Chem. 27:1569, 1955.
3. Parsons, J., Beher, W. T., and Baker, G. D.: Steroid x-ray diffraction powder data, Anal. Chem. 28:1514, 1956.
4. Parsons, J., Beher, W .T., and Baker, G. D.: Steroid x-ray diffraction powder data, Anal. Chem. 29:762, 1957.
5. X-ray Powder Data File, published by the American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pennsylvania.
6. Parsons, J., Beher, W. T., and Baker, G. D.: X-ray diffraction powder data and index for the steroids, Henry Ford Hosp. M. Bull. 6:365, 1958.

TABLE I

Index to Steroid X-Ray Diffraction Powder Data

Pattern No.	Name	Molecular Formula	Melting Point (Uncorr.), °C.
	C_{18} STEROIDS		
1	$\Delta^{1,3,5}$-Estratriene-3, 17α-diol	$C_{18}H_{24}O_2$	223-225
2	$\Delta^{1,3,5}$-Estratriene-3, 17α-diol-3-benzoate	$C_{25}H_{28}O_2$	194-196
3	$\Delta^{1,3,5}$-Estratriene-3, 17β-diol-17α-ethynyl	$C_{20}H_{24}O_2$	179-181
4	$\Delta^{5(10)}$-Estrene-11β-ol-3-one-17-ethynyl	$C_{20}H_{26}O_2$	180-184
5	19-Nor-Δ^4-androstene-17β-ol-3-one	$C_{18}H_{26}O_2$	113-115
6	19-Nor-Δ^4-androstene-17β-ol-3-one-17α-ethynyl	$C_{20}H_{26}O_2$	204-207
7	19-Nor-Δ^4-androstene-17β-ol-3-one-17α-methyl	$C_{19}H_{31}O_2$	152-155
	C_{19-29} STEROIDS		
	Monofluoros		
8	5α-Androstane-11β, 17β-diol-3-one-9a-fluoro-17*a*-methyl[a]	$C_{20}H_{31}O_3F$	199-201
9	Δ^4-Androstene-11β, 17β-diol-3-one-9α-fluoro-17*a*-methyl	$C_{20}H_{29}O_3F$	296-300
10	Δ^4-Androstene-17β-ol-3, 11-dione-9a-fluoro-17α-methyl[b]	$C_{20}H_{27}O_3F$	224-226
11	Δ^4-Pregnene-3, 20-dione-21-fluoro[b]	$C_{21}H_{29}O_2F$	144-147
12	Δ^4-Pregnene-17α-ol-3, 20-dione-6*a*-fluoro-17-acetate[c]	$C_{23}H_{31}O_4F$	263-267D
	Monohydroxymonoketones		
13	Androstane-3α-ol-17-one	$C_{19}H_{30}O_2$	185-187
14	Androstane-17β-ol-3-one-2α-methyl	$C_{20}H_{32}O_2$	150-152
15	$\Delta^{4,9,(11)}$-Androstadiene-17β-ol-3-one-17-methyl	$C_{20}H_{27}O_2$	171-173
16	Δ^4-Androstene-17β-ol-3-one-17α-ethynyl[a]	$C_{21}H_{28}O_2$	283-284
	Monohydroxydiketones		
17	5α-Androstane-17β-ol-3, 11-dione-17*a*-methyl	$C_{20}H_{30}O_3$	226-228
18	Δ^4-Pregnene-17α-ol-3, 20-dione-17*a*-caproate	$C_{27}H_{40}O_4$	118-120
19	Δ^4-Pregnene-17α-ol-3, 20-dione-6*a*-methyl-17-acetate	$C_{24}H_{34}O_4$	204-207
	Dihydroxymonoketones		
20	5α-Androstane-11β, 17β-diol-3-one-17*a*-methyl	$C_{20}H_{32}O_3$	149-151
21	Δ^4-Androstene-17β, 11β-diol-3-one-17-propionate[d]	$C_{22}H_{32}O_4$	150-153
22	Δ^4-Androstene-17β, 6β-diol-3-one-6, 17-diacetate	$C_{23}H_{32}O_5$	131-135
23	Δ^4-Androstene-17β, 11β-diol-3-one-17α-methyl	$C_{20}H_{30}O_3$	216-219
	Dihydroxytriketones		
24	Δ^4-Pregnene-17α, 21-diol-3, 11, 20-trione	$C_{21}H_{28}O_5$	228-231
25	$\Delta^{1,4}$-Pregnadiene-17α, 21-diol-3, 11, 20-trione	$C_{21}H_{26}O_5$	230-235
	Steroid Derivatives		
26	17a-Oxa-D-homo-Δ^4-androstene-3, 17-dione	$C_{19}H_{26}O_3$	212-216
27	17a-Oxa-D-homo-$\Delta^{1,4}$-androstadiene-3, 17-dione[c]	$C_{19}H_{24}O_3$	216-218

FOOTNOTES TO TABLE I

[a] Ethanol and ethyl ether
[b] Acetone and hexane
[c] Acetone
[d] Ethyl ether and hexane

TABLE II

X-Ray Diffraction Powder Data

1		2		3	
d, A	I/I_1	d, A	I/I_1	d, A	I/I_1
11.3	0.08	11.6	0.38	10.8	0.27
9.65	0.20	6.78	0.28	6.70	1.00
8.07	0.20	6.44	0.08	6.22	0.50
6.46	1.00	6.13	0.15	5.80	0.37
5.18	0.56	5.79	1.00	5.51	1.00
4.77	0.75	5.16	0.38	5.12	0.75
4.53	1.00	4.62	0.56	4.80	0.50
4.13	0.75	4.45	0.28	4.50	0.50
4.00	0.15	4.29	0.28	4.28	0.37
3.84	0.28	4.15	0.75	4.08	0.50
3.73	0.05	3.99	0.28	3.91	0.75
3.61	0.15	3.85	0.20	3.61	0.75
3.40	0.28	3.66	0.15	3.42	0.10
3.26	0.56	3.40	0.11	3.26	0.27
3.12	0.20	3.31	0.28	3.11	0.37
3.42	0.20	3.09	0.11	2.99	0.20
2.91	0.20	3.01	0.08	2.86	0.20
2.84	0.20	2.89	0.20	2.75	0.20
2.67	0.20	2.75	0.15	2.64	0.20
2.58	0.20	2.69	0.15	2.57	0.20
2.38	0.08	2.66	0.08	2.51	0.20
2.28	0.38	2.59	0.11	2.43	0.20
2.23	0.38	2.49	0.15	2.39	0.20
2.17	0.28	2.38	0.01	2.31	0.27
2.11	0.28	2.27	0.08	2.18	0.15
2.04	0.05	2.24	0.15	2.05	0.20
1.98	0.38	2.19	0.15	2.03	0.27
1.87	0.38	2.15	0.11	1.95	0.10
		2.09	0.11	1.90	0.10
		2.06	0.11	1.85	0.10
		2.04	0.11		
		2.00	0.05		
		1.96	0.15	6	
		1.86	0.11	d, A	I/I_1
		1.82	0.03	6.29	0.73
		1.78	0.03	5.81	0.73
		1.71	0.03	5.49	1.00
4		5		5.02	0.41
d, A	I/I_1			4.78	0.08
10.9	0.27	d, A	I/I_1	4.77	0.15
9.59	0.13	9.61	0.05	4.36	0.30
6.70	1.00	8.84	0.11	4.08	0.40
6.34	1.00	6.97	0.56	3.76	0.30
5.92	0.49	5.99	1.00	3.41	0.15
5.66	1.00	5.74	0.28	3.25	0.15
5.21	0.49	5.50	0.28	3.10	0.08
4.83	1.00	4.98	0.38	3.00	0.15
4.51	0.49	4.80	0.75	2.86	0.30
4.24	0.49	4.46	0.15	2.75	0.15
3.97	0.49	4.24	0.28	2.65	0.15
3.67	1.00	4.08	0.15	2.53	0.15
3.48	0.27	3.85	0.05	2.46	0.08
3.29	0.27	3.71	0.20	2.36	0.08
3.16	0.36	3.54	0.20	2.29	0.08
3.01	0.36	3.27	0.20	2.24	0.15
2.91	0.09	3.13	0.15	2.15	0.15
2.82	0.20	3.00	0.15	2.09	0.01
2.71	0.20	2.93	0.20	2.04	0.08
2.64	0.20	2.73	0.03	1.99	0.04
2.57	0.27	2.60	0.15	1.95	0.08
2.49	0.27	2.50	0.08	1.92	0.03
2.37	0.04	2.40	0.15	1.88	0.08
2.32	0.49	2.24	0.15	1.81	0.03
2.19	0.20	2.17	0.15	1.78	0.03
2.13	0.27	2.12	0.15	1.71	0.08
2.04	0.27	1.95	0.08	1.68	0.01
1.97	0.27	1.92	0.08	1.62	0.01
1.92	0.27	1.85	0.03		

7

d, A	I/I_1
8.91	0.38
8.28	0.38
7.09	0.15
6.47	0.15
5.97	1.00
5.41	1.00
5.14	0.56
4.73	0.56
4.56	0.20
4.20	0.28
4.04	0.28
3.84	0.15
3.56	0.20
3.42	0.20
3.26	0.15
3.11	0.15
3.03	0.20
2.95	0.15
2.89	0.28
2.69	0.28
2.58	0.11
2.45	0.11
2.33	0.28
2.20	0.28
2.15	0.28

8

d, A	I/I_1
12.3	0.15
11.2	0.05
8.65	0.05
7.75	0.41
6.83	0.41
6.53	0.27
6.19	0.73
5.62	1.00
5.14	0.41
4.94	0.11
4.78	0.11
4.54	0.11
4.36	0.05
4.22	0.11
4.03	0.05
3.91	0.05
3.76	0.27
3.54	0.20
3.37	0.04
3.19	0.11
3.23	0.11
3.08	0.08
3.00	0.05
2.87	0.02
2.78	0.08
2.65	0.08
2.50	0.05
2.48	0.11
2.43	0.11
2.35	0.04
2.27	0.04
2.22	0.04
2.16	0.05
2.10	0.11
2.04	0.05
1.96	0.02
1.89	0.02

9

d, A	I/I_1
11.1	0.20
9.77	0.07
7.97	0.75
6.19	0.15
5.83	0.37
5.64	1.00
5.47	0.27
5.04	0.37
4.63	0.37
4.36	0.27
4.23	0.37
3.93	0.10
3.75	0.27
3.47	0.07
3.36	0.15
3.22	0.10
3.13	0.10
2.98	0.20
2.93	0.27
2.84	0.15
2.73	0.20
2.66	0.20
2.55	0.10
2.48	0.20
2.33	0.20
2.27	0.15
2.17	0.07
2.13	0.10
2.07	0.10
2.04	0.15
2.00	0.03
1.93	0.10
1.85	0.02
1.81	0.02
1.67	0.10

10

d, A	I/I_1
10.4	0.56
6.44	0.75
5.91	0.38
5.32	0.75
5.10	1.00
4.61	0.11
4.47	0.28
4.10	0.28
3.93	0.28
3.73	0.11
3.45	0.20
3.23	0.20
3.06	0.20
2.96	0.20
2.83	0.20
2.69	0.28
2.61	0.28
2.57	0.28
2.46	0.08
2.41	0.28
2.32	0.15
2.28	0.15
2.22	0.15
2.17	0.20
2.10	0.05
2.05	0.20
1.97	0.20
1.97	0.20
1.93	0.20

11

d, A	I/I_1
8.23	0.15
6.89	0.27
6.51	0.41
6.22	0.27
5.74	0.41
5.02	1.00
4.48	0.15
4.15	0.15
3.96	0.08
3.75	0.55
3.55	0.08
3.41	0.08
3.33	0.08
3.13	0.20
2.93	0.20
2.79	0.04
2.74	0.08
2.69	0.08
2.53	0.11
2.48	0.08
2.33	0.15
2.18	0.11
2.07	0.11
2.04	0.11
1.96	0.11

12

d, A	I/I_1
9.64	0.38
7.71	0.08
7.04	0.05
6.43	0.75
5.57	1.00
4.99	0.15
4.79	0.38
4.35	0.11
4.20	0.38
4.05	0.11
3.91	0.11
3.58	0.11
3.42	0.38
3.19	0.38
3.06	0.20
2.97	0.03
2.73	0.05
2.67	0.11
2.65	0.11
2.58	0.11
2.48	0.05
2.45	0.15
2.38	0.15
2.30	0.11
2.25	0.11
2.19	0.20
2.12	0.20
2.03	0.11
2.00	0.20
1.94	0.03
1.88	0.15
1.80	0.20
1.77	0.20

13

d, A	I/I_1
10.9	0.11
7.28	0.11
6.76	0.40
5.97	1.00
5.64	0.40
5.04	1.00
4.71	0.15
4.28	0.30
4.10	0.15
3.88	0.15
3.76	0.20
3.69	0.11
3.49	0.11
3.37	0.11
3.20	0.08
3.14	0.25
2.94	0.15
2.79	0.11
2.66	0.04
2.57	0.11
2.45	0.20
2.38	0.20
2.28	0.01
2.23	0.01
2.19	0.15
2.15	0.15
2.10	0.15
2.00	0.01
1.96	0.06
1.93	0.06
1.90	0.06

14

d, A	I/I_1
11.9	0.15
8.29	0.38
7.33	0.28
6.25	1.00
5.62	0.38
5.19	0.38
4.92	0.75
4.59	0.15
4.32	0.15
3.95	0.75
3.69	0.11
3.51	0.11
3.39	0.20
3.23	0.11
3.11	0.08
2.99	0.20
2.77	0.15
2.69	0.01
2.48	0.11
2.41	0.11
2.36	0.11
2.31	0.11
2.22	0.11
2.18	0.11
2.09	0.05
2.02	0.05
1.96	0.05
1.91	0.05
1.84	0.05

15

d, A	I/I_1
10.8	0.03
6.72	0.03
6.12	1.00
5.88	0.47
5.46	0.75
5.24	0.08
5.02	0.28
4.64	0.11
4.33	0.56
4.05	0.56
3.84	0.15
3.74	0.20
3.49	0.20
3.35	0.20
3.27	0.11
3.18	0.11
3.06	0.28
2.94	0.15
2.80	0.11
2.75	0.38
2.66	0.15
2.63	0.15
2.58	0.08
2.51	0.08
2.43	0.20
2.38	0.11
2.31	0.08
2.25	0.15
2.17	0.20
2.12	0.01
2.05	0.05
1.99	0.20
1.92	0.05
1.86	0.05
1.83	0.01
1.78	0.01
1.74	0.15
1.68	0.15

16

d, A	I/I_1
6.30	1.00
6.03	0.75
5.36	0.50
5.05	1.00
4.66	1.00
4.20	0.20
4.05	0.27
3.85	0.10
3.67	0.15
3.49	0.37
3.27	0.03
3.13	0.37
3.02	0.37
2.87	0.37
2.71	0.27
2.60	0.02
2.52	0.27
2.43	0.27
2.34	0.07
2.26	0.01
2.19	0.37
2.11	0.07
2.05	0.20
2.00	0.20
1.93	0.10
1.88	0.03
1.82	0.03
1.79	0.10
1.75	0.10
1.63	0.03
1.51	0.03

17

d, A	I/I_1
10.9	0.15
6.15	0.73
5.43	0.20
4.85	1.00
4.50	0.20
4.11	0.20
3.92	0.10
3.76	0.08
3.57	0.11
3.13	0.08
3.04	0.08
2.95	0.15
2.77	0.01
2.67	0.03
2.58	0.08
2.51	0.11
2.43	0.11
2.34	0.04
2.25	0.11
2.21	0.04
2.17	0.08
2.09	0.08
2.06	0.15
1.99	0.08
1.93	0.01
1.87	0.01
1.84	0.06

18

d, A	I/I_1
12.2	0.56
8.93	0.20
7.14	0.38
6.24	0.38
6.11	1.00
5.40	0.28
5.02	0.38
4.50	0.38
4.29	0.05
3.98	0.15
3.83	0.15
3.61	0.15
3.45	0.05
3.25	0.15
3.04	0.08
2.86	0.08
2.78	0.08
2.63	0.03
2.57	0.03
2.48	0.05
2.36	0.08
2.30	0.08
2.18	0.08
2.10	0.11
2.03	0.11
1.98	0.05
1.93	0.05

19

d, A	I/I_1
10.8	0.05
9.12	0.20
7.53	0.20
6.86	0.41
6.28	1.00
5.31	0.27
4.78	0.41
4.54	0.20
4.36	0.27
4.21	0.15
3.78	0.20
3.45	0.20
3.29	0.20
3.15	0.05
3.01	0.05
2.73	0.05
2.68	0.04
2.61	0.04
2.54	0.11
2.49	0.04
2.43	0.04
2.36	0.04
2.27	0.11
2.21	0.04
2.17	0.04
2.12	0.04
2.07	0.08
2.06	0.02
2.01	0.04
1.94	0.04
1.90	0.02
1.85	0.02
1.82	0.04

22

d, A	I/I_1
11.6	0.08
8.38	0.15
7.11	0.20
6.56	0.20
5.57	1.00
4.91	0.11
4.35	0.30
4.10	0.11
3.90	0.20
3.72	0.11
3.53	0.20
3.36	0.20
3.19	0.11
3.04	0.08
2.75	0.15
2.57	0.04
2.49	0.15
2.34	0.01
2.18	0.20
2.10	0.01
2.04	0.01
2.01	0.04
1.94	0.03
1.90	0.03

20

d, A	I/I_1
7.94	0.49
6.28	0.09
5.66	1.00
5.36	0.27
5.02	0.36
4.71	0.27
4.52	0.36
4.26	0.36
3.83	0.27
3.71	0.27
3.08	0.09
2.98	0.27
2.83	0.13
2.70	0.20
2.64	0.27
2.56	0.09
2.49	0.02
2.35	0.13
2.25	0.04

23

d, A	I/I_1
10.7	0.15
10.2	0.08
6.99	0.15
6.94	1.00
5.73	0.27
5.55	0.27
5.32	0.27
5.06	0.08
4.43	0.41
4.17	0.08
3.83	0.08
3.60	0.11
3.47	0.08
3.35	0.20
3.17	0.20
3.11	0.20
2.96	0.20
2.86	0.20
2.83	0.20
2.71	0.11
2.66	0.11
2.60	0.08
2.54	0.11
2.50	0.08
2.46	0.20
2.36	0.15
2.31	0.15
2.28	0.15
2.23	0.15
2.19	0.15
2.14	0.02
2.10	0.02
2.08	0.08
2.04	0.08
2.01	0.01
1.90	0.01
1.89	0.11

21

d, A	I/I_1
9.31	0.03
7.83	0.20
6.61	0.37
6.17	0.27
5.59	0.27
5.19	1.00
4.54	0.20
4.32	0.15
4.04	0.20
3.79	0.20
3.63	0.15
3.43	0.03
3.24	0.07
3.08	0.07
2.95	0.07
2.82	0.03
2.61	0.03
2.49	0.03
2.37	0.07
2.25	0.07
2.15	0.10
2.03	0.10

24

d, A	I/I_1
7.37	0.11
6.07	1.00
5.46	0.15
4.85	1.00
4.62	0.08
4.24	0.08
3.97	0.08
3.70	0.40
3.43	0.30
3.30	0.20
3.07	0.20
2.95	0.15
2.86	0.30
2.73	0.01
2.72	0.08
2.51	0.08
2.42	0.08
2.36	0.08
2.28	0.08
2.21	0.15
2.12	0.11
2.06	0.11
1.98	0.11
1.94	0.06
1.91	0.06
1.86	0.01
1.81	0.03
1.79	0.01
1.72	0.01
1.67	0.01
1.64	0.01

25

d, A	I/I_1
7.52	0.07
6.10	0.75
5.45	0.20
4.92	1.00
4.61	0.20
4.24	0.20
4.02	0.20
3.69	0.27
3.60	0.15
3.39	0.27
3.29	0.15
3.06	0.27
2.96	0.15
2.86	0.27
2.78	0.03
2.50	0.15
2.39	0.15
2.33	0.03
2.22	0.07
2.16	0.07
2.11	0.07
2.05	0.15
1.90	0.07
1.78	0.07

26

d, A	I/I_1
8.05	0.50
6.66	0.27
6.20	0.20
5.30	1.00
4.94	0.50
4.41	0.50
4.27	0.02
4.04	0.10
3.80	0.50
3.66	0.20
3.47	0.20
3.29	0.20
3.17	0.20
3.11	0.27
2.98	0.10
2.86	0.20
2.79	0.02
2.70	0.02
2.59	0.10
2.45	0.02
2.38	0.02
2.27	0.20
2.01	0.07
1.94	0.03
1.89	0.03
1.83	0.07
1.81	0.02

27

d, A	I/I_1
8.76	0.03
7.80	0.56
6.68	0.28
6.28	0.15
5.68	0.11
5.37	1.00
4.93	0.75
4.66	0.05
4.37	0.38
4.09	0.15
3.92	0.28
3.74	0.56
3.48	0.11
3.31	0.15
3.18	0.15
3.05	0.08
2.95	0.20
2.87	0.11
2.69	0.03
2.58	0.20
2.44	0.08
2.35	0.01
2.26	0.20
2.20	0.20
2.15	0.08
2.11	0.08
2.05	0.03
1.97	0.15
1.92	0.11
1.87	0.15
1.78	0.11
1.70	0.08

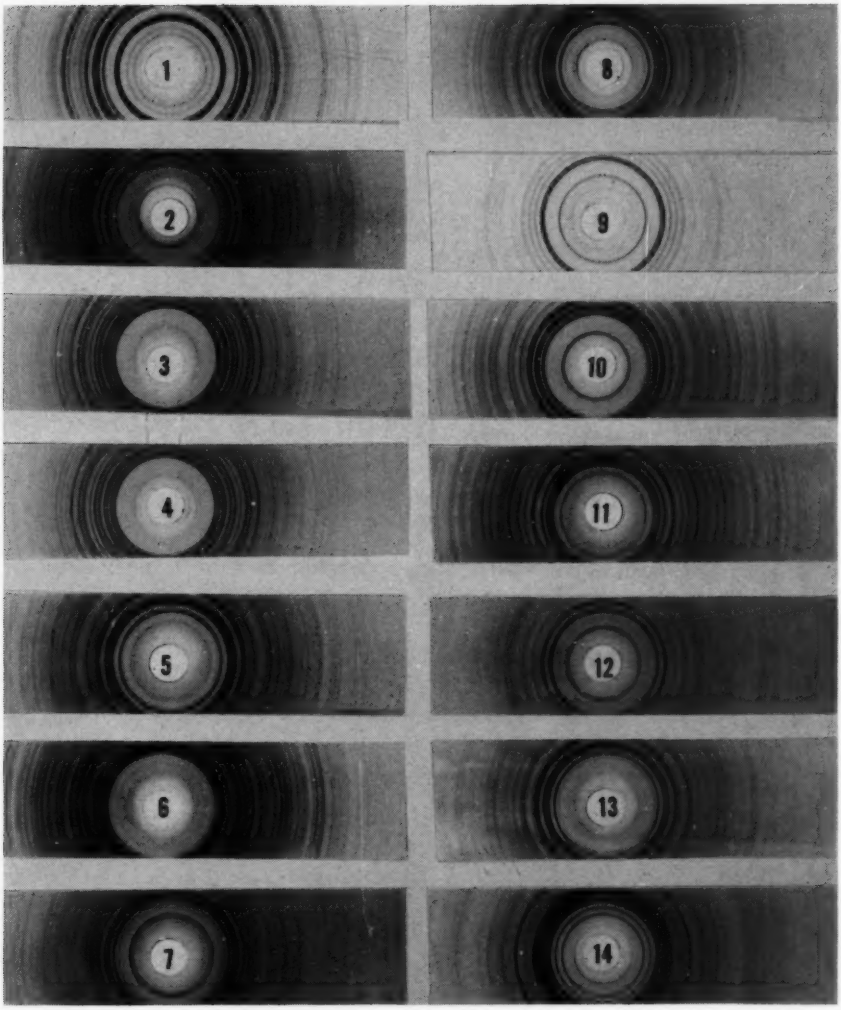


Figure 1a

X-ray diffraction powder patterns of steroids; pattern numbers 1-14. Key found in Table I.

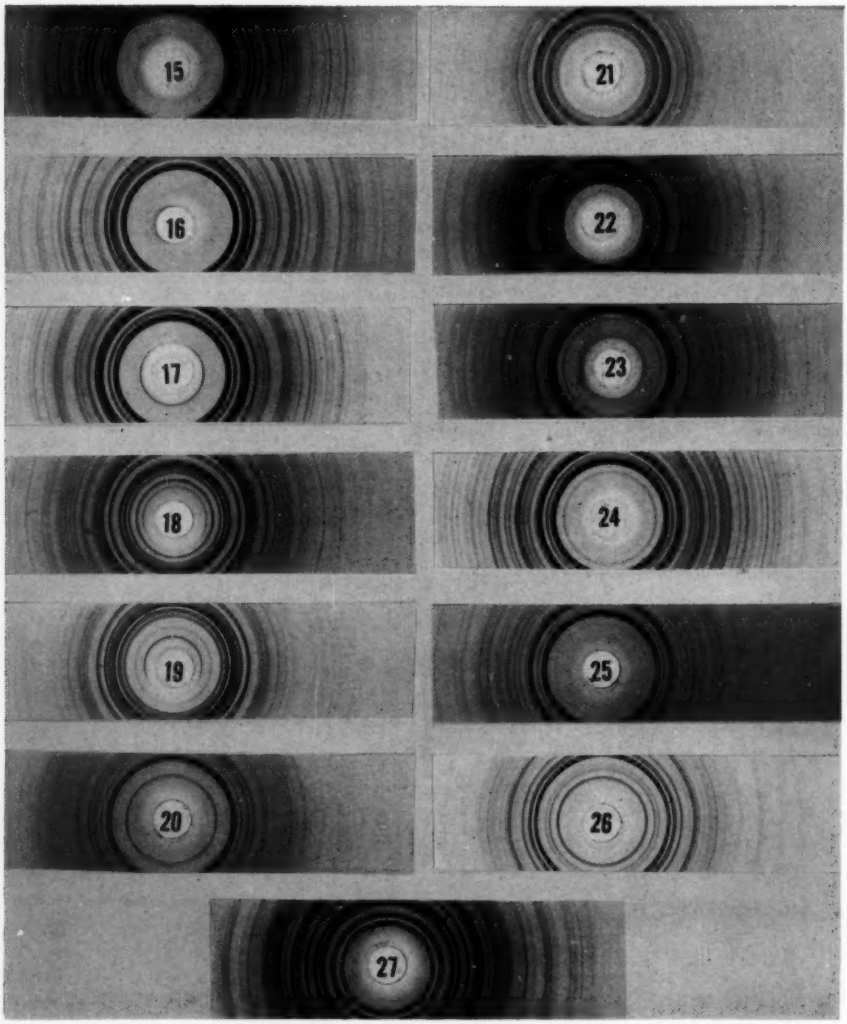


Figure 1b

X-ray diffraction powder patterns of steroids; pattern numbers 15-27. Key found in Table I.

ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

A CLINICAL SURVEY OF A CROSS-SECTION OF AN INDUSTRIAL WORKER GROUP. R. E. Birk. Arch. Environmental Health 1:291, 1960.

One of the main problems concerning the value of periodic health examinations on supposedly well patients is whether or not the yield of treatable conditions will be high enough to warrant the expenditure of time and money. The present study would seem to put an end to any concern regarding this point. The average of 3.2 diagnoses per patient, 7.9% requiring surgical correction and 66.0% requiring medical therapy, alone warrants such examinations.

NEVUS SEBACEUS WITH ASSOCIATED BASAL-CELL EPITHELIOMA. M. Brannen and R. P. Fosnaugh. Arch. Dermat. 82:1007, 1960.

Nevus sebaceus is a hamartoma composed primarily of mature sebaceus glands. Apocrine glands are frequently found lying beneath the masses of sebaceus glands. Abortive hair follicles may be present. It is not uncommon to have a basal-cell epithelioma develop within a nevus sebaceus. The tumor is usually present at birth. It is commonly located on the scalp or face. However, it may occur at other sites, such as the neck. It is usually a single, linear, well-circumscribed firm lesion. The lesion is raised above the skin surface and is yellow in color. Incomplete removal will result in recurrence, or the development of nevus syringadenomatosus papilliferus. For this reason surgical excision is the treatment of choice.

MEGALOBLASTIC ANEMA ASSOCIATED WITH INHIBITION OF THYMINE SYNTHESIS (Observations during 5-fluorouracil treatment). M. J. Brennan, V. K. Vaitkevicius and J. W. Rebuck. Blood 16:1535, 1960.

Profound hematopoietic depression often occurs as a result of intensive treatment with 5-fluorouracil. Morphologic cellular studies of bone marrow before, during, and after administration of the drug have been undertaken in all but a few of 200 patients given this agent in the Oncology Division, Henry Ford Hospital, during the past 18 months. Clinical results of this chemotherapy are reported elsewhere. Megaloblastic changes in the erythrocytic series and cellular giantism in the myeloid line characterize the bone marrow response to 5-fluorouracil in man. Cytoplasmic vacuolizations occur in reticulo-endothelial cells, megakaryocytes, and myeloid elements. During development of these morphologic changes in marrow, associated cellular giantism, polynucleism, and nuclear pattern changes appear in the oral, vaginal, colonic mucosa, and in some tumors resembling those encountered in the bone marrow. The myeloid-erythroid ratio was found to be useful for the purpose of identifying dosage end-points. The physiological implications of these changes as related to nucleic acid metabolism are discussed.

DIFFERENTIAL DIAGNOSIS OF ACUTE PAIN IN THE UPPER ABDOMEN. B. E. Brush. Canad. M. A. J. 83:925, 1960.

In the diagnosis of acute pain in the upper abdomen, one must use every available facility and, in addition, a good bit of thoughtful consideration of the symptoms

and physical findings in order to arrive at a correct diagnosis. A very careful study of the nature of the pain, its onset and its radiation and character must all be considered, since most diseases cause pain which is rather typical for them. Determination of blood cell counts, temperature, and pulse and respiratory rates and a radiograph of the abdomen may add confirmation to the diagnosis and permit the physician to determine whether the condition is a surgical emergency, or whether the patient should be kept under close observation for a time and have the progress of the disease carefully observed. It is advisable to think first of many conditions and then to narrow to a conclusion, instead of thinking about some abdominal condition and trying to fit the patient's symptoms into this picture. In many instances the old axiom that "one must think of the condition before it can be diagnosed" is true of diseases which cause pain in the upper abdomen. In our experience it has been helpful to think first of the organ involved and then to try to delineate the nature of the pathological reaction. The young physician should develop a plan for approaching these acute problems, if his diagnostic acumen is to be developed to its fullest. The ability to interpret the information available on a patient increases with knowledge and experience. If he accepts each problem as a challenge and uses his knowledge and common sense, his percentage of accurate diagnoses will be great, and his experiences gratifying.

*THE ULTRASTRUCTURE OF THE TAIL OF THE CERCARIA OF *HIMASTHLA QUISSETENSIS* (Miller and Northup, 1926). R. R. CARDELL, JR. AND D. E. PHILPOT. Tr. Am. Microscop. Soc. 79:442, 1960.

Cercariae of *Himasthla quissetensis* were obtained in the free-swimming form, fixed in 1% osmium tetraoside, sectioned on an ultramicrotome and studied with an electron microscope. The structure and ultrastructure of the tail were observed and described in detail. The tail is enclosed by a cuticle which contained small mitochondria and vacuoles. Internal to the cuticle is a layer of smooth muscle running at right angles to the longitudinal axis of the tail. Below the smooth muscle is a layer of striated muscle with its fibers directed obliquely. Associated with the striated muscle are many large mitochondria. The center of the tail is composed of parenchyma cells.

THE "DUMPING" SYNDROME. L. S. FALLIS AND J. BARRON. Société Internat. de Chir. XVIII Congrès, 1959. Bruxelles, Imprimerie Medicale et Scientifique, 1960, p. 587.

There is general agreement that approximately 15 percent of patients submitted to subtotal gastrectomy for ulcer do not obtain maximum results from the operation. Patients in this category may be classified into three groups, viz., those who should not have been subjected to surgical intervention because of emotional instability or a pre-existing nutritional problem, those with recurrent ulceration, and those who suffer from the (dumping) syndrome. The majority of patients with this unfortunate complication of subtotal gactric resection for ulcer have their symptoms relieved by appropriate medical treatment and many of them improve with the passage of time. There is, however, a small group of patients whose symptoms do not abate and their condition deteriorates to the point that they suffer from such an advanced degree of malnutrition that they are unable to engage in any activity that requires sustained

*Edsel B. Ford Institute for Medical Research.

effort. If vagotomy has not been previously done it should be the preliminary step of the operation. The technique employed in the individual case depends on whether the patient has had a Billroth I or a Billroth II gastrectomy. Our preliminary experience with a limited number of patients during the past three years has been good, but its real value will be manifested only after more time has elapsed. We attribute our good results to: Discriminatory selection of patients for operation, preoperative preparation by restoration of nutritional balance by means of forced tube feeding, and complementary vagotomy on patients with high acid values.

*THE NATURE OF THE FLUORESCENCE OF AN ENZYME-REDUCED COENZYME-REDUCED SUBSTRATE COMPLEX. H. F. FISHER AND L. L. MCGREGOR. Biochim. Biophys. Acta 43:557, 1960.

This paper presents evidence that L-glutamate decreases the dissociation of the glutamic dehydrogenase-TPNH complex, and that the increase in the intensity of fluorescence of that complex caused by the addition of L-glutamate is due solely to the increase in the amount of TPNH bound rigidly to the enzyme surface.

HYPERTENSION DUE TO RENAL INFARCTION; REPORT OF A CASE. M. Z. FRUCHTMAN AND J. R. CALDWELL. New England J. Med. 263:907, 1960.

A case of unilateral renal infarction with no apparent cause in a thirty-nine-year-old woman is presented. Acute, established diastolic hypertension developed and was not responsive to moderate drug treatment but was cured by nephrectomy. Examination of the involved kidney revealed massive but subtotal necrosis. The blood pressure has remained within the normal range for more than a year after operation.

THE DIRECT METHOD OF ANTIBIOTIC SUSCEPTIBILITY TESTING OF STAPHYLOCOCCI. J. E. GREER AND R. R. MENARD. Am. J. M. Technol. 26:249, 1960.

An adaptation of the procedure of *Bondi et al* has been used to test the antibiotic susceptibility of staphylococci in 596 primary cultures from the skin and nares. In the 372 skin cultures producing sufficient *S. aureus* for testing, 96% yielded the same results when the organisms were tested in pure culture. Results in the remaining 4% were unsatisfactory because of masking by resistant normal flora and spreading gram negative rods. Knowledge of the cultural characteristics of the organisms involved, however, prevented incorrect interpretation of the results. In a few cases, (7%) the procedure had the additional advantage of permitting recognition of staphylococcal strains with different antibiograms but similar morphology. Results with nasal cultures were similar.

UNIQUE MORPHOLOGIC FEATURES OF WHIPPLE'S DISEASE. W. S. HAUBRICH, J. H. L. WATSON AND J. C. SIERACKI. Gastroenterology 39:454, 1960.

The distinctive morphologic feature of Whipple's disease is not the accumulation of fats emphasized as "intestinal lipodystrophy," but rather it is the ubiquitous occurrence of large, sickle-form particle containing (SPC) cells which are probably derived from the recticuloendothelial system. Characteristic SPC cells are abundant in the lamina propria of the small intestine and in the mesenteric lymph nodes, but their

*Edsel B. Ford Institute for Medical Research.

identification in the tissues of all major organ systems confirms that Whipple's disease is a systemic affection. The periodic acid-Schiff (PAS)-positive sickle-form particles may constitute a phase in the cytoplasmic elaboration of an abnormal protein-carbohydrate complex by the SPC cells. It is suggested that the SPC cells of Whipple's disease may represent a widespread proliferation by a metabolically aberrant strain of mutant reticular cells.

ELECTROCARDIOGRAPHY FOR NON-ELECTROCARDIOGRAPHERS. T. N. JAMES. M. Times 88:1455, 1960.

Not requiring meticulously detailed knowledge of electrocardiography, the majority of physicians are faced either with the task of tedious study of a book on a subject they will not employ very often, or of remaining uninformed about it. In the belief that these particular physicians might find useful a resume of what information an electrocardiogram, optimally interpreted, might be expected to provide, this brief review is presented. To be clear, this perspective discussion is not to make electrocardiographers of non-electrocardiographers. It is not to sell electrocardiograms, if there is no information to gain from them. By describing some of the infomation obtainable by careful interpretation of an electrocardiogram, it is hoped it can be made a more useful laboratory examination for those who do not have frequent recourse to it.

DIURETIC THERAPY OF CONGESTIVE HEART FAILURE. J. W. KEYES. J. Michigan M. Soc. 59:1674, 1960.

In this article are reviewed the xanthines, the cytosines (aminouracils), the carbonic anhydrase inhibitors, the organo-mercurials, the thiazides (benzothiadiazines), phthalimidine and steroids. The stepwise advancement in the treahment of congestive heart failure allows the use of various diuretic materials. One can select a drug to fit almost every stage and expect reasonable success. Combinations of these agents also may give better response under certain conditions. The natural history of the abnormal pathologic physiology involved in congestive heart failure makes possible the proper selection of a diuretic. Diuretic drugs should be continuously evaluated in order to obtain the greatest benefits from their use.

THE USE OF THE NEWER DIURETICS IN THE TREATMENT OF CARDIAC DISEASE. J. W. KEYES AND G. M. BRENEMAN. Ann. New York Acad. Sc. 88:915, 1960.

Experience with the different thiazide analogues has shown them all to be exceptionally good diuretic drugs. All diuretic drugs are adjunctive in the control of edema in cardiac failure and are never used to supplant digitalis, salt restriction, and reduced physical activities. Diuretic effectiveness may fail for various reasons; however, electrolyte depletion and failure to maintain adequate glomerular filtration rate seem to be chiefly responsible. While unusual side effects of these drugs do occur, and they may seem to have been overemphasized here, the conclusion remains that they may occur but are infrequent. They are presented to call attention to possible causes of trouble that may seem inexplicable to the practitioner unaware of their potentialities. The use of corticosteroids and aldosterone-blocking agents in refractory heart failure has been discussed for the purpose of pointing out that with the adjunctive use of thiazides or mercurials diuresis may be restored. It is felt that the thiazide drugs

approach the mercurials in potency, but do not exceed them except, because of their low toxicity and repetitive action, in controlling and stabilizing the edema state in long-term diuretic therapy.

THE BEHAVIOR OF C-REACTIVE PROTEIN IN THE CASE OF MYOCARDIAL INFARCTION. G. LAUBINGER AND E. M. PRIEST. Klin. Wchnschr. 38:662, 1960.

(Translation of the summary by N. Waehneldt, M.D.)

The C-reactive protein is a pathological protein structure which develops in the blood subsequent to inflammatory, necrotic and neoplastic reactions. It occurs in 88-100% of cases of acute intramural myocardial infarction (91% in our series). It can usually be demonstrated on the second, or at least on the third day after onset of the disease. During the third week it is usually not demonstrable any more in the blood stream. The C-reactive protein test is more valuable than the sedimentation rate insofar as the CRP test is earlier demonstrable than a rise of the sedimentation rate and, on the other hand, with subsiding of the inflammatory processes, the CRP test also disappears more rapidly. However, both tests are unspecific as to the myocardial infarction. Compatible observations with C-reactive protein, sedimentation rate, white blood count and serum transaminase on 59 patients with acute transmural and 43 patients with non-transmural cardiac infarctions are described.

ILLUSTRATING MEDICAL SUBJECT MATTER. W. LOECHEL AND R. M. MORRELL. Med. Ann. District of Columbia 29:498, 1960.

Medicine's spectacular advances of the last century have been marked by a concomitant evolution of the paramedical sciences. It is important that each field properly explain its mission. Seeing all, as does the camera, is often undesirable. Gross dissections of cadaver material or operating room procedures usually result in a multitude of unnecessary highlights, drapery, hands, etc. The camera cannot think or know how to superimpose two layers of related information succinctly in one picture. Also, the publishing of many photographs costs more and uses excessive space. For the greatest economy of method in the removal of excess "visual baggage" the illustrator's talents are invaluable.

INTUSSUSCEPTION FOLLOWING INTESTINAL INTUBATION. W. J. MERSCH AND M. A. BLOCK. Arch. Surg. 81:887, 1960.

The occurrence of small-bowel intussusception related to intestinal intubation with the long intestinal tube is recorded in 2 patients. Both were successfully managed. This rare complication of intestinal intubation should be kept in mind when the long tube is used. If a long intestinal tube has been inserted before surgery and appears to be intussuscepting on small bowel at the time of surgery, correction of this condition may prevent a later complication.

NEPHROGENIC POLYCYTHEMIA. R. K. NIXON, W. O'ROURKE, C. E. RUPE AND D. R. KORST. Arch. Int. Med. 106:797, 1960.

Three aditional cases of nephrogenic polycythemia involving cystic lesions of the kidney are presented. Two of these cases are unique in that simple excision of the cysts produced remission of the polycythemia. High levels of erythropoietin activity were demonstrated in the cyst fluid and cyst wall of one case. The clinical and ex-

perimental literature pertaining to the subject of nephrogenic polycythemia is briefly reviewed, and a tentative hypothesis relating to the tubular cell in the pathogenesis of this disorder is offered. It is hoped that a more widespread knowledge of this entity will lead to the proper diagnosis and treatment of both its hematologic and renal aspects.

THE VENTILATORY RESPONSE TO CARBON DIOXIDE IN MITRAL DISEASE. H. G. PAULI, F. E. NOE AND E. O. COATES. Brit. Heart J. 22:255, 1960.

The regulation of ventilation in 7 patients with pure or predominant rheumatic mitral stenosis was investigated by measuring respiratory response to increasing CO_2-content of the inspired gases. Respiratory responsiveness, as defined by increase in specific ventilation produced by a given increase in arterial CO_2-tension, was found to be significantly lowered in comparison with 11 normal subjects. On the other hand, the extrapolated central threshold value for the arterial CO_2-tension was diminished in these patients, resulting in a slight rise of ventilation at the level of resting arterial PCO_2. Two patients were studied before, and 84 and 111 days respectively after, successful mitral valvotomy. Their respiratory response was found to be increased after operation, while the central arterial PCO_2 threshold was raised. The possible mechanisms of these changes in respiratory regulation are discussed.

BEHAVIORAL CHANGES DURING HYPOTHALAMIC OR LIMBIC STIMULATION IN THE MONKEY. L. D. PROCTOR, R. D. KNIGHT, J. LUKASZEWSKI AND J. BEBIN. Am. J. Psychiat. 117:511, 1960.

Stimulation of various areas of the limbic and hypothalamic regions in the Macaca mulatta resulted in a variety of responses. These responses were compared with those obtained on stimulating areas of the mid-brain reticular formation. It would appear that "overlapping" occurs as to the function of the limbic system, the mesencephalic reticular formation and the hypothalamus, in view of the similarity of behavior patterns elicited by stimulating some areas in these three different regions. The EEG patterns from the limbic, reticular (mesencephalic) and hypothalamic systems varied significantly so as to suggest that each system may function basically as an individual component system of the brain, probably influenced by ramifications one with the other. This EEG study is a preliminary investigation.

ACOUSTIC TRAUMA OF THE COCHLEA FROM EAR SURGERY. H. F. SCHUKNECHT AND J. TONNDORF. Laryngoscope 70:479, 1960.

It is well known that high energy acoustic signals, such as those created by a blast in air or a blow to the head, can cause cochlear damage having its maximal effect in the upper basal turn. Such lesions can also be created by manipulation of the stapes during the course of surgical procedures. The amplitudes of such maneuvers can be very large and the time constants are long. The resulting cochlear injuries are identical to those resulting from stimuli having short time constants. Animals with experimentally produced footplate fractures frequently sustain rupture of the saccule, as well as injury to the upper basal and lower middle turns of the cochlea. Saccular rupture also occurred in one of ten animals subjected to head blows. The cause for the saccular injury is not clear. A new concept is developed concerning the mechanism of acoustic trauma. The assumption is made that the ensuing tissue damage is a function of the physical stress resulting from acoustic stimulation. Experiments on cochlear

models using transient signals indicate that the closer the stress is concentrated to the basal end of the cochlea the shorter is the time constant of the applied signal. Since the sensitivity of the model falls off for signals with very short time constants, a region of optimal stress develops close to but not directly at the extreme basal end of the cochlea. It is possible that signals of relatively long time constants, but of very large amplitudes, produce injurious stresses in the basal region of the cochlea due to non-linear distortion.

COMMENTS ON TYMPANOPLASTY. H. F. SCHUKNECHT, T. M. MCGEE AND S. OLEKSIUK. Laryngoscope 70:1157, 1960.

Reconstructive middle ear surgery is usually unsuccessful in the presence of the Eustachian tube malfunction, hyalinized collagen deposits, and deficient mucous membrane; ordinarily it should not be performed for patients over 60 years of age. Skin graft failures can occur immediately, or many months after surgery. Graft failures can be decreased by the proper selection of the donor site, careful handling and approximation of the graft to the recipient site, use of cutting burrs only, and by controlling suppuration. Tympanoplasty will achieve and maintain a position of respect among otological procedures only if we, as surgeons, properly assess the underlying surgical pathology and achieve appropriate microsurgical skills.

STAPEDECTOMY. H. F. SCHUKNECHT, T. M. MCGEE AND B. H. COLMAN. Ann. Otol. Rhin. & Laryng. 69:597, 1960.

The results with stapedectomy are so good that in spite of the incidence of delayed cochlear degeneration, the operation appears superior to other procedures for otosclerosis. At this time we believe there is no functional difference between various implants being used to replace the stapes, so the choice of technique is one of individual preference. A factor of great importance is that of surgical skill. The skilled surgeon, following strict rules of procedure to avoid labyrinthine trauma, will experience fewer complications than the occasional operator. Of 287 stapedectomies in which a tissue-metal implant was used, bone-air gap of 10 db or less was acquired in 74.9%. Delayed cochlear degeneration occurred in 2.4%. On the basis of earlier experiences, we predict that bony reclosure of the oval window will occur eventually in ears in which there is extensive or active otosclerotic disease. On the other hand, we believe that the operation may be permanently successful for ears with small inactive otosclerotic lesions.

BRONCHIAL ASTHMA IN ADULTS. J. H. SHAFFER, L. L. DILELLA AND J. A. MARVEL. J.A.M.A. 174:1810, 1960.

Adults with bronchial asthma present a complex problem. Heredity, atopy, and food allergy diminish while inhalant allergy, infection, and degenerative changes increase in importance. Cystic fibrosis has been encountered with increasing frequency in adults and should be considered as a possible cause in wheezing. Treatment plans for bronchial asthma should include measures to relieve bronchospasm, reduce edema and evacuate mucus from the bronchi, control allergenic factors, treat infection when it is present, and improve the general health of the patient. Concomitant cardiovascular changes in the elderly asthmatic patient require careful selection and dosage of drugs of the sympathomimetic group. The palliative effect of steroids, through their anti-

inflammatory action on the allergic reaction, is well documented. Such preparations should, however, be used in conjunction with a well-rounded program in the treatment of bronchial asthma.

THE CONTRIBUTION OF ANGIOPLASTIC SURGERY TO THE THERAPY OF PERIPHERAL OCCLUSIVE ARTERIOPATHY: A CRITICAL EVALUATION OF EIGHT YEARS' EXPERIENCE. D. E. SZILAGYI, R. F. SMITH AND J. G. WHITCOMB. Ann. Surg. 152:660, 1960.

An attempt has been made to evaluate the record of performance of 485 angioplastic operations (mainly grafting) in 385 patients followed clinically and angiographically for periods up to eight years. The immediate postoperative patency rate was best in the treatment of aortoiliac lesions and, in general, in the less-advanced forms of disease. The patency rates declined in time in all categories but most markedly in the femoro-popliteal operations, where the patency fell to 17 per cent in five years. The deterioration in the aorto-iliac group was much less marked and never fell below 60 per cent. The ratio of limb loss to limb salvage was satisfactory. The mortality rate was acceptable. An excellent rate of work rehabilitation was achieved. The good results of aorto-iliac operations encourage their continued widespread use. The disquieting fact must be kept in mind, however, that in the most advanced forms of disease these procedures have an appreciable rate of mortality. Since calf claudication due to segmental femoral occlusive lesions is essentially a benign disease, and since the operative morbidity for its surgical treatment is not negligible, in view of the poor late results the use of grafting procedures in its treatment should be restricted to a small group of cases in which the symptoms are truly disabling or which show rapid progression.

DIAGNOSIS AND SURGICAL TREATMENT OF INTRACARDIAC MYXOMA AND RHABDOMYOMA. R. E. TABER AND C. R. LAM. J. Thor. Cardiov. Surg. 40:337, 1960.

Primary cardiac tumors were considered a pathologic curiosity until the development of open-heart surgical techniques. The most frequently encountered tumor is the myxoma which arises in approximately three fourths of the cases from the left side of the atrial septum. Symptoms and findings of mitral stenosis or insufficiency may be produced by a left-sided tumor and closely resemble those of rheumatic valvular disease. Changing murmurs, positional aggravation of symptoms, refractory heart failure, and history of arterial embolization in the presence of minor cardiac findings are clinical features which may assist in the differentiation. Chest roentgenograms, the electrocardiogram, and cardiac catheterization support a diagnosis of disease at the mitral valve but positive identification depends upon angiocardiography. Right-sided atrial myxoma is most likely to be confused with lesions producing a high right atrial pressure, such as rheumatic tricuspid stenosis, intractable arteriosclerosis heart failure, constrictive pericarditis, or Ebstein's malformation of the tricuspid valve. Here again, positive diagnosis depends upon use of angiocardiography. Two patients are presented who had successful removal of right atrial myxomas using cardiopulmonary bypass. One patient demonstrated a right-to-left shunt preoperatively which was most pronounced in the left lateral position. Shunting occurred through an interatrial septal defect. Approximately 30 minutes of extracorporeal perfusion were required in both

cases to remove the tumor, excise the site of septal attachment, and repair the resulting defect. The myxoma characteristically has a superficial attachment without invasion of the underlying myocardium and most likely is a neoplasm rather than a thrombus. Primary rhabdomyoma of the heart has heretofore been described as a rare incidental necropsy finding in infants. The tumors are unencapsulated and may be multiple or solitary. They have been found in association with tuberous sclerosis, adenoma sebaceum, and visceral hamartomas and are regarded as a developmental abnormality rather than a true neoplasm. A case is reported in which a ventricular septal rhabdomyoma produced outflow tract obstruction in the right ventricle. The obstructing portion of the tumor was successfully excised during cardiopulmonary bypass. The patient, a child 7 years of age, exhibited some of the signs of tuberous sclerosis. Preoperative diagnosis and surgical treatment of primary cardiac rhabdomyoma have not previously been reported.

IN DEFENSE OF EPONYMS, AND A COMMENT ON "A PROPER ST. LOUISAN"

A. B. SCHWARTZ, M.D.

MILWAUKEE, WISCONSIN

Stevens-Johnson is as terse as time
And clear cut as culebra
Beside "Ectodermosis Erosiva Plurofacials" of Von Hebra.

Mnemonically a desert
No visceral strings — Gadzooks!
But audiophonically more apt than
"Syndroma mucocutaneo-oculare acutum" of Fuchs.

The only fault of eponyms —
Rediscovery breeds begats.
But not when alert indexers
Keep out contenders' hats.

On gastroenterospasms
Dulled memory casts a blight.
It would be simpler to recall
The syndrome of Park White.*

What matters when redeemed of sin
What symptom complex done you in?
The pearly gates will set you free
From syndrome and from syndromé.

**Am. J. Dis. Child.* 26:91, 1923.

Reprinted with permission from the New England J. Med. 262:1029, May 19, 1960. This poem is reprinted because of its close bearing on Robert H. Durham's book, "Encyclopedia of Medical Syndromes", New York, P. B. Hoeber, 1960.

DUPLICATE

HENRY FORD HOSPITAL MEDICAL BULLETIN

ORTHOPEDIC ISSUE

Vol. 9 March, 1961 No. 1
Part II

CONTENTS

1. Author's Foreword. i

   HAROLD M. FROST, M.D.

2. Human Osteoblastic Activity. Introduction. 75

   HAROLD M. FROST, M.D.

   I. A Comparative Method of Measurement With Some Results. 76

   HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA, B.S.

   II. Measurement of the Biological Half-Life of Bones With the Aid of Tetracyclines. 87

   HAROLD M. FROST, M.D.

   III. The Effect of Cortisone on Lamellar Osteoblastic Activity. 97

   HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA, B.S.

   IV. The Effect of Severe Cardiac Failure. 100

   HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA, B.S.

3. Feathering: A Theory of Genesis. 103

   HAROLD M. FROST, M.D.

4. A Possible New Cause for Aseptic Necrosis. 115

   PHILIP H. MCFARLAND, M.D., AND HAROLD M. FROST, M.D.

5. Halo Volume. Introduction to Qualitative Studies. 123

   HAROLD M. FROST, M.D.

   I. The Effect of pH on Size. 126

   HERBERT ROTH, B.A., HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA. B.S.

   II. The Effect of Citrate Ion Concentration on Size. 130

   HERBERT ROTH, B.A., HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA. B.S.

   III. Existence of a Pattern in the Matrix. 133

   ANTHONY R. VILLANUEVA, B.S., HAROLD M. FROST, M.D., AND HERBERT ROTH, B.A.

(Contents continued on inside back cover)

HENRY FORD HOSPITAL
MEDICAL BULLETIN

March 1961 — Part II

FURTHER PAPERS FROM THE ORTHOPEDIC RESEARCH LABORATORY*

by

HAROLD M. FROST, M.D.,
Associate Surgeon, epartment of Orthopedic Surgery

ANTHONY R. VILLANUEVA, B.S.,
Research Assistant, Orthopedic Research Laboratory

HERBERT ROTH, B.A.,
Senior Medical Student, Wayne State University, College of Medicine

and

PHILIP H. MCFARLAND, M.D.,
Former Resident, Department of Orthopedics. Present Address, Fullerton, Calif.

*Earlier papers of this series appear as March 1960 Part II of the BULLETIN.
The work presented in these papers was supported by Grant No. 293, Henry Ford Hospital Research Funds.

AUTHOR'S FOREWORD

It is a simple fact that when a man publishes a professional paper, the value thereof accrues to his name and reputation. The value may be positive or negative, depending upon how the work is judged by his peers and by the merciless insight which time provides.

Men whose vision is restricted by far, rather than near, horizons know that this strictly personal valuation is, with few exceptions, false. It is false because it omits more than it embraces.

The individual doing research is surrounded by material and ghostly factors which exert a profound influence on the results of his research.

Research cannot be done effectively in the face of an indifferent or hostile administration, department head, or attitude on the part of adjuvant departments and services, no matter how determined nor how able the investigator. The consequence is that when a good piece of work is done equal credit accrues to the institution from which the work comes. More specifically the credit accrues to the department head responsible for the investigator, and to the administration whose policies provide the backing and atmosphere that are crucial to effective research.

In other words effective research is a cooperative effort. While the investigator and his institution contribute differently both deserve credit for the result.

The work has come out of the Henry Ford Hospital Orthopedic Research Laboratory is beginning to attract favorable attention beyond the walls of the hospital. Some of the new concepts introduced into skeletal physiology and pathology have been found useful by several authorities in explaining experimental data. Some of the measurements done in the laboratory have been found useful by others who are attempting to inject some quantitive base into our knowledge of bone.

This is gratifying. It must be pointed out, however, that the work referred to could not have been done by the individuals concerned, alone. Henry Ford Hospital, its policy making groups, and Dr. C. Leslie Mitchell, Chief, Department of Orthopedic Surgery, are as much responsible for the work as any individual. There are few department heads and few medical centers that provide such able and consistent support to orthopedic basic research. There are few pathology departments who have contributed so much material and been so willing to supply the type of material necessary for the type of work done in the laboratory.

It is a unique and self-evident tribute to Henry Ford Hospital and its personnel that these things exist in an institution devoted to the private practice of medicine.

HAROLD M. FROST, M.D.

HUMAN OSTEOBLASTIC ACTIVITY

INTRODUCTION

HAROLD M. FROST, M.D.

It has long been known that in order to measure that portion of turnover of the human skeleton due to constant resorption of old bone and laying down of new bone, methods were needed to permit dealing with resorption and formation on a separate basis. In practice this proved difficult to achieve, the reasons being complex, largely technical, and more than enough to account for previous difficulty.

Since the conclusion of World War II numerous attempts have been made to measure bone formation by using various radioactive isotopes. Many of these attempts merit being called achievements instead of attempts, since the values they derived are significant and are the correct order of magnitude. Turnover measurements as done by men such as G. C. H. Bauer in Sweden, and C. Nordin in Britain, are in the writer's mind as these comments are made.

The basic difficulty with such measurements is well appreciated by the men who are making them. Radioactive calcium, strontium, and other isotopes are not only deposited in the skelton in places where new bone is being added, but are also deposited in some parts of the large mineral mass in the so-called resting skeleton. Accumulating evidence suggests that this mineral mass may be divided into at least two and perhaps three compartments simply on the basis of the rates with which exchange between the bone mineral and blood may occur. As a result it is difficult to say how much of a retained isotope is deposited in new bone and how much is deposited in already existing bone. This is the nubbin of the problem of measuring bone accretion with radioactive isotopes.

This problem may be restated as follows: tracer methods need a quantitative frame of reference.

In the following series of four papers, and in some of the preceding work which will be referred to in these papers, we present measurements of osteoblastic activity in a sizeable series of human beings. It has been possible to state the amount of new bone formed per unit volume of bone per unit time. The methods serve as cross checks on each other and as a result the writer is confident of the validity of the measurements, the methods, and inferences or conclusions drawn from them. There *is* one drawback in the methods, in that the measurements are limited to a few bones in the skeleton. Most of the measurements in fact have been done on ribs, clavicles, and femurs. Cancellous bone and the bones in the axial skeleton apart from rib have largely been ignored.

To achieve a quantitative statement of new bone formation in the entire skeleton, these deficiencies must obviously be dealt with in some manner.

The other side of the turnover equation, that of bone resorption, will be dealt with by a separate and subsequent series of publications.

HUMAN OSTEOBLASTIC ACTIVITY — PART I

A COMPARATIVE METHOD OF MEASUREMENT WITH SOME RESULTS

HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

Measurement of human osteoblastic activity has long been the dream of skeletal physiologists. The dream has escaped conversion to reality because of the existence of formidable technical problems and lack of basic physiological knowledge. Recent developments in this and other laboratories now make such measurements possible. This paper is the culmination of work done to provide these measurements. Some results are presented which, in conjunction with companion papers, cover measurements made on bones from over 150 patients.

Figure 1

Cross section, clavicle, human, undecalcified, basic fuchsin. An Haversian system lies in the center of the field. The central Haversian canal is outlined at its wall by the inner array of India ink marks. Between the inner and outer array of India ink marks lies an osteoid seam, elaborated by osteoblasts during the formation of this Haversian system. Accordingly this is a new Haversian system, formed as a part of the body's normal remodelling skeletal activity.

The center of the Haversian system is occupied by somewhat shrunken vascular and connective tissue elements. The Haversian system is about three-fourths completed. Formation proceeds in centripetal, rather than in centrifugal, fashion.

There are numerous reasons for wanting measurements of human osteoblastic activity, apart from the obvious ones of curiosity and the wish to measure the previously unmeasurable. Three of these reasons will be briefly discussed in the following paragraphs.

A) Osteoporosis

In general, it is known that bone is formed only through the activity of cells known as osteoblasts, and destroyed only through the activity of cells known as osteoclasts*. If osteoporosis is defined as bone with thinner cortices and trabeculae, and fewer trabeculae than normal, and if it is assumed that the osteoporotic skeleton was once normal, then there must have been more osteoclastic activity than osteoblastic activity to produce this situation. There was a negative skeletal balance, in other words. A change in skeletal balance** can occur in one of five generically possible ways. These are (1) unequal increase in accretion and osteoclasia, (2) unequal decrease in accretion and osteoclasia, (3) complete cessation of accretion or, (4) of osteoclasis, (5) increase of one and decrease in the other mode of activity.

Four of the five possible types of alteration of skeletal balance listed could cause osteoporosis. There are no existing quantitative measurements which point out which of the four possibilities actually is responsible, for example, for senile osteoporosis. The current *theory* is that osteoblastic activity is largely controlled by the protein anabolic hormones, that the decrease in output of these hormones with advancing age is accompanied by decrease in osteoblastic activity without alteration of osteoclastic activity, and that this is the cause of the osteoporosis.[1,2] In spite of this theory, patients seldom correct the skeletal defect on anabolic hormone treatment, and some even get worse. This indicates a lack in the theory of the genesis of osteoporosis of old age.[11] Measurements of osteoblastic activity are needed to inject a core of fact into this problem and to suggest which of the possible mechanisms mentioned is the cause of the osteoporosis.

B) Interpretation of in vivo Balance Studies

Many methods of measuring calcium balance are known and in use. Broadly speaking their purpose is to determine if more bone is being formed than destroyed during the epoch of the study. It is usually assumed, in the absence of evidence to the contrary, that a positive calcium balance occurs as the result of an excess of bone accretion over osteoclasia, and conversely. It can now be stated with some assurance that this need not be so.

For example, assume a steady-state skeleton - i.e.: one in which the total amount of bone matrix is not changing, and in which formation equals destruction. Theoretically a positive calcium balance in such a situation may occur as the result of halo volume accretion,[15] progressive mineralization of feathered bone,[8] progressive mineralization of relatively young but incompletely mineralized bone, and crystallite adsorption or exchange.[18] On the other hand, a negative balance could occur theoretically from halo volume and crystallite elution. These factors, and others perhaps unknown, plus non-skeletal changes not considered here, could operate simultaneously.

So many unmeasured variables (including osteoblastic and osteoclastic rates) makes interpretation of balance studies in which these variables are ignored a very

*A former controversy over this point should be laid to rest because conclusive evidence documenting this statement now exists, supplied by several independent laboratories.
**Not to be confused with Calcium balance.

unreliable process. Measurement of osteoblastic activity would enable us to place a quantitative estimate on at least one of these variables. From this would be learned the relative importance of the remaining variables. They might be minute or overwhelming.

C) Radioisotope Studies

When radiocalcium, radiosodium, or other skeletally depositing isotopes are given to animals or man, and the skeletally retained moiety subsequently determined,[3,4] several variables can theoretically combine to explain an observed effect. These factors have been mentioned: feathering,[8] micropetrosis,[7] halo volume,[15] mineralizing new matrix, osteoclasia, and at more fundamental levels, factors governing diffusion impedence between crystallite and the blood supply,[9] and factors governing the solubility and activity coefficient of the bone crystallites.[18] Almost no attempt has been made to quantate the first 7 factors named, partly because most of them are new knowledge, and partly because there were no feasible methods available. The number of variables eloquently portrays how essential quantitation of one or more of them is in order to assign quantitative significance to the remainder.

THEORY OF MEASUREMENTS OF SEAMS/MM^3

A brief review of some material published by the Orthopedic Research Laboratory will orient the reader of this paper. Details will be found in the references listed.

1) Properly stained fresh, undecalcified, cross sections of the diaphyses of long bones were utilized in this work. The sections average about 35 microns thick, comprise the entire cross sections of the bone and are cut accurately perpendicular to the longitudinal axis of the bone.[5,6]

2) Osteoid seams may be quantitatively stained and quantitatively recognized *only* in sections prepared as recommended above.[13]

3) Longitudinal homogeneity, transverse inhomogeneity, has been found to characterize the skeletal distribution of a number of features, among them osteoid seams, feathering, micropetrosis and tetracycline labelled new bone formation.[14] This orientation has obvious effects on the type of sampling and sections that are needed.

4) Only measurements on diaphyses are reported. Measurements of cancellous bone can be made but correction factors must be applied due to lack of uniform orientation. These correction factors are laborious and subject to large errors.

The restriction of material to diaphyses means that the values of osteoblastic activity cannot be considered values for the entire skeleton or entire bones, since cancellous bone might have different activities than cortical bone. It has already been noted that significant differences occur in activities between the axial and appendicular moieties of the skeleton.

It is possible that in the future a normalization factor can be determined which will enable measurements of one bone to be normalized to the entire skeleton.[3,4] This would remove the present, bothersome restriction on interpretation of the present data.

5) Osteoblastic activity sounds fine, but how to measure it? Should one measure oxygen consumption per osteoblast, new matrix formed per unit volume or the number of osteoblasts per osteoid seam? We have elected to measure the number of osteoid seams per unit volume of cortex. Such a measurement does not reveal the rate of new bone formation, and does have inherent in it a number of possible defects which will be discussed later. Seams per unit volume are easy to measure, are reproducible, and when averaged in sufficient number provide a smooth curve of variation with age.

6) In a previous publication it has been noted that osteoid seams are normally present in human adults as well as in children.[12] In the present 500 cases in the laboratory's files there are no cases in which seams are not found except in some cases of severe cardiac failure, some cases of severe hepatic cirrhosis or in most patients receiving large doses of cortisone or ACTH. Included in the case material are 90 healthy, normal people dying of violence, embolism, sudden hemorrhage, or coming to surgery for the correction of anatomic defect rather than systemic disease. These cases exhibit measurements lying in the median range of the curves in Graph I.

7) The actual method of doing the measurements is detailed in reference (13) which was published specifically to detail this part of this paper. The measured parameter is the number of seams/mm^3.

8) It should be noted that only osteoid seams *in* the cortex are measured in this work. Endosteal and periosteal seams are not measured. It should also be noted that the normal, lamellar bone remodelling processes that occur in childhood and adulthood are always accompanied by the presence of seams at sites of new bone formation.[12] A peculiar, resting state of osteoid seams induced by abnormal factors has been eliminated as a variable in this work by excluding resting seams from counts.[10]

MATERIALS

Sixty-two ribs and 52 clavicles from 93 patients were measured. Males comprise 62% of the cases. Thirty of the ribs were obtained fresh from the operating room from thoracotomies. The remainder of the material was obtained at postmortem.

Hindsight has been exercised in determining which cases to include and which to exclude from this study. The following have been *excluded:* any patient on cortisone or related compound or ACTH for more than 2 weeks prior to skeletal sampling; any patient on androgens or estrogens; any patient with severe hepatic cirrhosis; any patient with severe congestive heart failure; any patient with known local disease of the bone obtained; any patient with systemic bone disease, including multiple myeloma and skeletal metastases; any patient with disturbed serum calcium, phosphorus or alkaline phosphatase; any patient with symptomatic osteoporosis.

METHODS

1) Fresh, undecalcified, cross sections were made and stained as recommended by Frost.[5,6] Section thickness averaged about 35 microns and should not exceed 50 microns.

2) The number of osteoid seams per unit volume was measured as described in reference (13) and expressed as seams per mm^3.

3) The measurements were then tabulated in groups according to the age of the patient, the divisions in age being made every 5 years. Totally separate tabulations were made for the ribs and the clavicles.

4) The average values for a given age — say age 60 — were made by averaging the measurements from all cases 5 years younger, and all cases 5 years older, than the given age. The average figure for age 60 then is the mean of the measurements of all cases aged 55 to 65 in the series. This method of obtaining values for a curve has the advantage that the curve is a smooth one and thus easy to grasp. It has the disadvantage that possible actual short time fluctuations in values are averaged out. Until the number of "normals" is larger by a factor of about 5, it will be impossible to be sure whether short-term fluctuations were real or due to chance.

5) The values for rib and calvicle are plotted separately, using a linear scale for age and a logarithmic scale for seams/mm^3.

6) A discussion of errors is necessary to place the values in perspective. Empirically it has been determined that the measurement of channel/mm^2 varies plus or minus 0.005 and the seam counts plus or minus 0.01. With the optical constants specified in reference (13) there is an additional constant, proportional error in seam counts because about 3% of them are too thin to be recognized at the magnification and numerical aperture used. Shrinkage of undecalcified cross sections is about 2% in the radial and tangential axes (longitudinal shrinkage can be completely ignored in the present measurements).[13]

There is no error due to failure to demonstrate an osteoid seam, and there is no error due to failure to recognize an osteoid seam, except for the proportional error referred to previously.

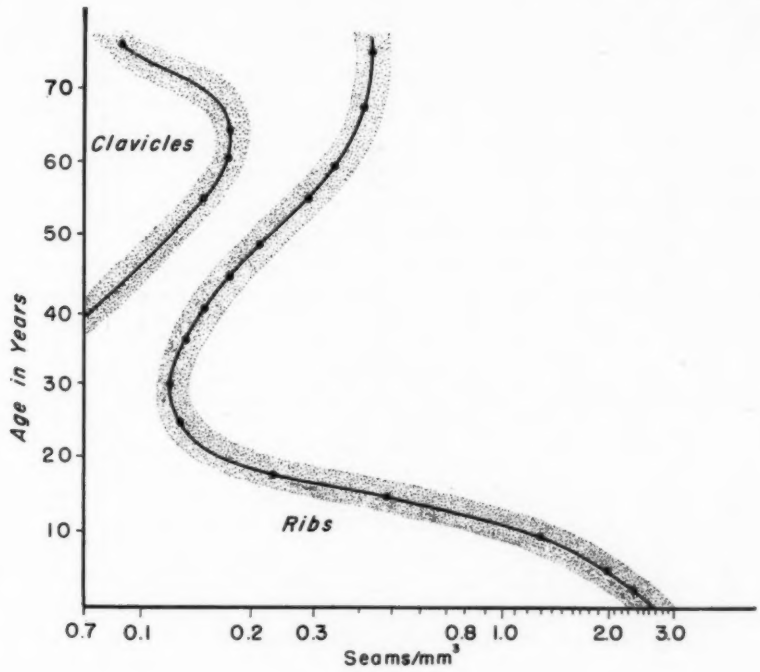
All the above errors combined are so small in comparison to the actual fluctuations in seams/mm^3 in different cases that correction for them is superfluous. In other words, the sensitivity of the method exceeds the needs of the task.

The major source of possible error is in the number of sections measured in each case.[13] A total of 191 rib sections and 98 clavicle sections were measured, an average of about 3 rib sections and two clavicle sections per bone examined. The variations found in single bones will be reported separately.[13] Suffice it to say that measurement of one rib section carries a probable error in the neighborhood of 100%; of 3 rib sections about 20%; of two clavicles about 10%. Since in some cases only one section was measured, and in others 7 or 8, the reliablility of the individual measurements varies widely. Since the scatter in values has repeatedly proved to be real rather than a sampling defect, we have resorted to the device of lumping cases together to minimize the scatter and to permit graphing the changes in osteoblastic activity with age.

OBSERVATIONS

The mean values of seams/mm^3 for both rib and clavicle will be found in

Graph I. The characteristic curve should be noted, and the fact that over the majority of their courses the curves of the two bones parallel each other. Note the sharp decrease in osteoblastic activity near age 30 and the fact that it gradually increases thereafter. A decrease after age 30 is manifested only in the clavicle and only after age 65. Note that, with the exception of some variation in the 30-40 age range, the curves obtained are smooth, indicating that the number of cases measured and sampled is sufficient for the present purpose. If the number were too small, there would be sizable sinusiodal variations along the majority of the curve.



Graph 1

The curves are drawn around the mean values plotted by 5-year age groups. The curve of the ribs is to the right, that of clavicles to the left. There is no curve for clavicles below age 40 because local undertakers object to the deformity resulting when a part of this bone is removed in younger clients.

Age is the abcissa on a linear scale. Seams/mm³ of diaphyseal cortex is the ordinate on a logarithmic scale. The curve as depicted is artificially smoothed.

In Table I the mean values of the various rib age groups plotted in Graph I are listed along with the number of cases in each group. Since there is considerable overlap in these group mean determinations (see Methods) they do not total out to the total number of cases available.

RIBS

Mean Age	Mean Value Seams/mm³	Number Cases Averaged
2	2.40	3
5	1.96	7
10	1.27	5
15	.47	4
20	.23	6
25	.12	8
30	.12	8
35	.15	9
40	.16	10
45	.17	9
50	.20	9
55	.30	11
60	.33	11
67	.43	7
75	.42	8

Table I

CLAVICLES

Mean Age	Mean Value Seams/mm³	Number Cases Averaged
40	.077	4
55	.151	17
60	.184	21
65	.195	17
70	.115	14
75	.092	8

Table II

All of the cases in this study have been placed in 5-year age groups and tabulated in these columns. Ribs and clavicles are tabulated separately. The age of the group is on the left, the mean value of the group in the middle, and the number of patients in the group in the right columns.

In Table II the mean values of the various clavicle age groups are similarly listed.

In Table III some individual age groups are listed, each case in the group and its sex being noted. The purpose of this is to permit the reader to see for himself the amount of scatter to be expected in these measurements.

The values of seams/mm³ in young children is probably deceptively low, the reason being that endosteal wall osteoid seams were not counted in the measurements.

A considerable number of such seams are to be found in the bones of rapidly growing children.

RIBS			CLAVICLES		
Age Group	Sex	Value Seams /mm³	Age Group	Sex	Value Seams /mm³
46-50	M	.28	56-60	M	.29
	F	.22		M	.10
	F	.25		M	.36
	F	.158		M	.095
				F	.23
51-55	M	.33		F	.08
	M	.28		M	.053
	M	.149		F	.087
	M	.54		F	.11
	M	.29		M	.02
56-60	M	.314	61-65	M	.20
	M	.80		M	.12
	M	.093		M	.358
	M	.53		F	.35
	M	.34		M	.037
	F	.56		M	.75
				M	.17
61-65	M	.147		M	.156
	F	.258		M	.04
	F	.25		F	.11
	M	.088		F	.16
	M	.11			

Table III

4 AGE GROUPS FROM THE SERIES OF RIBS, AND TWO FROM THE SERIES OF CLAVICLES, are presented here in detail. Some idea of the scatter may be obtained from inspection of the individual measurements. There is no clear-cut difference between the sexes.

DISCUSSION

1) As was intimated earlier, saying that osteoblastic activity will be measured is fine, but when the task materializes the investigator must decide exactly how osteoblastic activity will be measured. In general one might measure some aspect of metabolic activity, or the total amount of matrix formed in unit time, or the total amount of mineral deposited in unit time, or the total volume or number of osteoid seams formed per unit time. There are other possibilities but the point is made — the task seems complex.

2) This laboratory has been very fortunate in having available (a) simple methods for preparation of fresh, undecalified sections which contain less artifact than bone sections made by any other existing method; (b) possessing the largest collection of such sections in the world with complete clinical data on hand on every case; (c) in possessing material from a large number of cases to whom tetracyclines were administered at varying times and durations prior to skeletal sampling; (d) in possessing superiors with a true desire and understanding of the need for basic research.

With these advantages a study such as the present one is possible. The most likely phenomenon that could be used to measure osteoblastic activity at first appeared to be the osteoid seam, which had already been observed to be normal, rather than abnormal, accompaniment of skeletal maturity and senescence.

3) Measuring osteoblastic activity in terms of seams per unit volume possesses certain possible faults which had to be checked. These faults, and the manner in which they were dealt with, are briefly:

A) The possibility that new matrix is mineralized so quickly that it is not present in sufficient thickness to be detectable optically. This was checked using tetracycline-labelled material in which the tetracycline had been administered within two weeks of skeletal sampling.[16,17,14,12] The tetracycline-labelled new bone always corresponded to the presence of an osteoid seam. No tetracycline-labelled new bone existed without an accompanying seam in this material.

B) The possibility that the rate of production and mineralization of new osteoid varies with age, as well as the measured variation in number per unit volume. In other words, there is a possibility that a decreased measured value of seams/mm^3 might correspond to *increased* osteoblastic activity because the few seams present are being formed and mineralized much more rapidly than would be normal at another age. While variations in rate of formation do occur, increases of rate are accompanied by increases in seams/mm^3.* If the resting state of osteoid seams is compensated during the counts,[10] then it is also true that retardation in rate of osteoid formation is accompanied by fewer seams/mm^3.* Finally, no systematic variation in rate, other than the high activity expected in children, has yet been detectable in tetracycline-labelled material. The predicted slowing up of osteoblastic activity in the years past age 40 were not only *not* observed; there is a slight increase in rate of formation of new osteoid with advancing age.

C) The possibility that osteoid seams are abnormal. This matter has been dealt with elsewhere but, in brief, this is a false notion. Seams are a normal finding at all ages. Absence of seams at any age seems to be evidence of severe disturbance in the body's physiology.

D) The possibility that cortical remodelling may show variations in age totally different from the variations in cancellous bone. At present there are no quantitative measurements which dispose of this point, only qualitative observations in a few tetracycline-labelled skeletons. While there are differences in rate between cancellous and cortical bone, their variations seem to vary in the same manner in the small amount of material observed to test this matter.

If this possibility should prove true — which is not likely — knowledge of such a skeletal characteristic should prove most valuable.

E) The possibility that clavicles and ribs are peculiar bones not representative of the skeleton as a whole. The laboratory possesses over 200 femora, tibias, fibulas, radii, ulnae, humerii, phalanges, metacarpals, metatarsals, vertebrae and iliac crest specimens. There is no essential difference between these bones and those reported

*Checked with tetracycline labelled cases.

on in this paper. Clavicles and ribs were selected for this study solely because enough of them are available and have been measured to derive significant mean values according to age.

4) It hardly needs to be pointed out that the nature of fluctuation of the curve of osteoblastic activity in Graph I is quite the opposite from that which would be expected from current theories about factors governing osteoblastic activity. It is widely felt that the protein anabolic hormones exert a considerable effect on osteoblastic activity, acting as an accelerator. It is further felt that with increasing age, after the 30 year decade, a progressive fall in the endogenous supply of protein anabolic hormones, plus a disproportionately smaller fall in the endogenous supply of protein catabolic hormones, leads to "defective matrix formation". The latter term is usually interpreted to men that a decreased amount of matrix is formed but that the quality of the matrix which is formed is normal.

The curves in Graph I do not agree at all with the above thesis. There are three possible reasons for this disagreement:

(a) The measurements in this paper are in error (they have been checked *most* carefully).

(b) Some quirk of osteoblastic physiology exists which has not been detected and which, when compensated for, will make the measurements presented in this paper compatible with the above-mentioned theory.

(c) The theory is wrong.**

These three possibilities are stimulating, and it is hoped that skeletal physiologists find them of interest.

SUMMARY

Measurement of human osteoblastic activity, expressed as osteoid seams per unit volume, has been done. Measurements on human ribs and clavicles are graphed according to age. The curves indicate that at birth osteoblastic activity is quite high, that it decreases rapidly until age 25-30 where it levels off, only to climb slowly but steadily until age 65. Beyond this age the significance of the curves is impaired by lack of numbers. It appears at present that ribs continue to reveal a relatively high osteoblastic activity beyond this age, but that clavicles show a dropping off in activity after this age.

The present methods and measurements are qualitative because the problem of *Volume of bone formed* per unit volume of bone *per unit time* has not been solved. Until such a solution is at hand the present measurements, while instructive and indicative of the quantitative nature of the variation of osteoblastic activity, cannot be interpreted in terms of so many milligrams of calcium per day retained, so much matrix nitrogen per day retained, so much turnover per day and so on.

**This is our present opinion.

REFERENCES

1. Albright, F.: Osteoporosis, Ann. Int. Med. 27:861, 1947

2. Albright, F., Bloomberg, E. and Smith, P. H.: Post-menopausal osteoporosis, Tr. A. Am. Physicians 55:298, 1940.

3. Björnerstedt, R., and Engstrom, A.: Maximum permissible body burden of Strontium-90, Science 129:327, 1959.

4. Eckelmann, W. R., Kulp, J. L., and Schubert, A. R.: Strontium-90. II. In man, Science 127:266, 1958.

5. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

6. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

7. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

8. Frost, H. M.: A new bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

9. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

10. Frost, H. M.: Observations on osteoid seams: The existence of a resting state, Henry Ford Hosp. M. Bull. 8:220, 1960.

11. Frost, H. M.: Osteoporosis: a hard look, J. Am. Geriatrics Soc. (In Press).

12. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

13. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. 35:179, 1960.

14. Frost, H. M., Villanueva, A. R., and Roth, H.: Tetracycline staining of newly-forming bone and mineralizing cartilage in vivo, Stain Technol. 35:135, 1960.

15. Frost, H. M., Villanueva, H. R., and Roth, H.: Halo volume, Henry Ford Hosp. M. Bull. 8:228, 1960.

16. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

17. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

18. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

HUMAN OSTEOBLASTIC ACTIVITY — PART II

MEASUREMENT OF THE BIOLOGICAL HALF-LIFE OF BONES WITH THE AID OF TETRACYCLINES

HAROLD M. FROST, M.D.

INTRODUCTION

A need exists for direct measurement of osteoblastic activity in man. The ideal measurement should quantate volume and time, and thus measure rate. A convenient means of expressing rate would be in terms of the biological half-life of a bone.* There are diverse reasons for wanting measurements of the biological half-life of bone. A cogent one is the need to place bone accretion in some quantitative perspective to other activities such as halo volume exchange, mineral accretion by feathered bone, ionic exchange or fixation at the surface of the bone crystallites,[15] and the normal diffusion impedence processes found in bone.[8]

In a previous paper measurements of osteoblastic activity in terms of osteoid seams per unit volume were presented.[8] While these measurements permit qualitative comparison of osteoblastic activity in various decades, they need a quantitative basis. A measurement of rate is needed to provide a quantitative basis and to allow a check on some of the variables discussed in that paper which might affect the validity of the interpretation of some skeletal physiological data.

The door to a new and enormously useful method of investigation of human bone physiology in vivo was opened when Milch, Rall, and Tobie published their basic observations concerning the fixation of the tetracycline antibiotics in bone.[13,14] From their observations, and the work of this laboratory, it has been learned that any of the current tetracycline antibiotics becomes fixed in mineralizing cartilage and mineralizing new bone; that this fixation is permanent; that there is no translocation of the tetracycline once so fixed; that tetracycline is detectable as long as 9 years after deposition in the skeleton; that fresh, undecalcified sections are necessary for detection and observations of the tetracycline; that the tetracycline can be seen with ordinary brightfield microscopy, given suitable preparation of the section;[9,10,11] that microfluorescence demonstrates the tetracycline in undecalcified bone;[4] that a simple and economical microfluorescence set-up exists;[4] that in newly forming, lamellar bone the "instantaneously staining zone" is a band about 4 microns thick;[6] that this band creeps centripetally about 0.9 microns per day; that the width of the resulting tetracycline "label" is the result of duration of dosage and speed of mineralization; that normally mineralized bone is impermeable in vitro and in vivo to the tetracyclines;[11] and that tetracycline labelling carries all of the advantages that would accrue from being able to give humans of all ages large doses of radiocalcium or radiophosphorus, but none of the disadvantages.

From the sizeable intellectual meal above, it can be seen that it should be possible to measure the bone formed during a period of tetracycline administration, and, if

*The biological half-life, as used here, is defined as the time required for the normal bone remodelling processes to remove and replace half the absolute bone volume, if they proceeded steadily at the measured rate.

one knew the duration of the drug administration, calculate a true rate of bone formation from the measurements. While there are several possible methods of making such measurements, in this laboratory light microscopy has been used. The method, and some results, are presented in this paper.

MATERIALS

Table I lists measurements made on 27 bones from 21 patients. The essential features of the patients are listed in Tables I and II. The cases were selected because

Table I

Case	Bone	Total Days Tetracycline (D	Epochs (E)	K_{ie}	V_{vasc} %	V_{lab} %	V_{lab}, abs, Corr. %	V_{lab}, abs, Corr. Month, %	Biological Half-Life of Bone Years	Months Between Labelling and Sampling
1.	Rib	24	2	.73	9.0	15.3	12.6	15.7	.27	.9
2.	Rib	28	1	.86	6.1	9.5	8.9	9.5	.48	.5
3.	Clavicle	231	6	.9	12.5	9.8	8.4	1.2	3.5	3.
4.	Clavicle	10	2	.53	6.2	.81	.47	1.4	3.0	9
5.	Clavicle	5	1	.53	16.5	.76	.49	3.0	1.4	.63
6.	Clavicle	6	1	.57	4.8	.92	.64	3.2	1.3	3.4
6.	Rib	"	"	"	8.7	.79	.51	2.5	1.6	"
7.	Rib	11	2	.55	8.6	.15	.093	.25	16.8	6
8.	Rib	3	1	.40	11.6	.116	.054	.54	7.7	20
8.	Clavicle	"	"	"	7.1	.12	.053	.53	7.8	"
9.	Femur	40	3	.75	6.5	.07	.057	.004	97	2.3
9.	Fibula	"	"	"	19.5	.35	.32	.0032	12.6	2.3
10.	Rib	14	2	.60	4.0	.073	.046	.09	46	.3
11.	Femur	91	9	.69	6.1	4.1	3.1	.94	4.4	19
12.	Clavicle	21	5	.49	8.1	.87	.47	.67	6.2	1.2
13.	Clavicle	16	2	.64	6.3	.34	.24	.45	9.3	37
14.	Femur	38	6	.59	9.4	.52	.39	.21	20.3	2.7
14.	Tibia	"	"	"	9.1	.098	.075	.04	105	"
14.	Fibula	"	"	"	5.2	.67	.49	.26	16.1	"
15.	Clavicle	16	1	7.8	—	0	0	0	oo	.5
15.	Rib	"	"	"	—	0	0	0	oo	"
16.	Clavicle	3	1	.40	—	0	0	0	oo	26
17.	Clavicle	10	1	.69	5.1	.048	.036	.11	38	.3
18.	Clavicle	16	2	.64	5.1	.33	.23	.43	10.6	1.5
19.	Clavicle	6	1	.57	5.6	.29	.177	.88	4.7	42
20.	Clavicle	3	1	.40	3.7	.18	.00048	.48	8.7	1.2
21.	Clavicle	5	1	.53	5.6	.21	.12	.72	5.8	109

Refer to Text.

at varying known times during their medical historical period at Henry Ford Hospital they received tetracycline antibiotics for known duration prior to skeletal sampling. Sampling means obtaining bone from the patient, either at surgery or at post mortem. Note that in most cases the osteoblastic activity labelled with tetracycline and measured, occurred at some time before the patient's death, last illness, or surgery. In inter-

pretation of such measurements it is necessary to know the medical history of the patient during the labelling epoch; the history subsequent to the labelling epoch is of little moment for most purposes.

The possibility occurs, in children particularly, that subsequent remodelling may remove part of the labelled bone, leading to measured values which are too low. Correction for such an event is easily computed, but is not done in the present series of cases. It would be indicated whenever the interval of time between labelling and sampling is greater than about 20% of the calculated half-life of the bone.

Table II

HFH Case	Case	Sex	Age	CHF	Cortisone or ACTH	No Meical Problem (Skeletally Normal)	Ischemia	Tetrallogy of Fallot	Osteoporesis, Severe	Wide Metastases	Diabetes Mellitus	Abnormal Ca,P Values	Normal Ca,P Values	O. R. Spec.	Post Mortem Spec.	COMMENT
95 82 06	1	M	0.5	X											X	Pneumonitis
94 77 60	2	F	0.5												X	Very ill
87 93 01	3	F	13		X			X							X	Cortisone addict
85 59 25	4	F	15		X				X						X	Rheumatoid Arthritis, Pulmonary Fibrosis
95 44 39	5	M	15											X		Mitral Stenosis
93 43 12	6	F	16												X	C. P.
82 26 04	7	F	35											X		Commissurotomy
20 95 34	8	M	39	X									X		X	CHF
98 27 40	9	M	43											X		Osteomyelitis Tibia, years
49 81 16	10	F	47			X								X		Adenoma Bronchus
83 68 47	11	M	53											X		G. C. T. Tibia
95 05 12	12	M	56		X					X					X	
06 20 70	13	F	56		X					X					X	
99 30 14	14	M	57				X						X	X		Aortic Graft
06 32 98	15	M	60		X										X	Leukemia
84 33 18	16	F	61		X				X			X			X	Rheumatoid Arthritis
89 07 93	17	M	62												X	CVA
19 89 63	18	M	64												X	Periarteritis
24 30 10	19	F	68							X			X		X	
94 30 71	20	F	68												X	Brain Tumor
21 10 77	21	F	73						X		X				X	Nephrosclerosis

Refer to Text.

METHODS

SECTIONS

Fresh, undecalcified sections were made, stained and mounted as recommended by the writer.[1,2] These methods seem to be essential for the clear and quantitative demonstration of labelled bone. Section thickness should be about 40 microns.

MICROSCOPY

Accurate measurement of tetracycline labelled bone necessitates examination by microfluorescence. A simple and economical method exists and is available to anyone who has the use of a medical microscope.[4] It serves as well as the orthodox but expensive equipment sold by the major microscope manufacturers. This set-up was utilized in making the measurements in Table I.

MEASUREMENTS

Measurements were made only on complete cross sections cut accurately perpendicular to the longitudinal axis of the bone. Sections are through the diaphyses of the bones examined. Measurement of cancellous bone is possible but necessitates correction factors for the lack of uniform orientation. The correction factor is difficult to determine.

A) *Longitudinal homogeneity; transverse inhomogeneity:* Under certain conditions area measurements on cross sections of diaphyseal bone supply values equivalent to the proportion of the volume of the total cortex composed of the measured phase. Characteristics such as feathering, micropetrosis, and osteoid seams per unit volume seem to follow the rule of "longitudinal homogeneity, transverse inhomogeneity." In such oriented material adequate sampling is obtained *only* from cross sections.[9] Labelled, new bone activity follows this rule, as would be expected in view of its relationship to osteoid seams and its probable relationship to feathering. Since there is far less variation along the length of the bone than in the transverse axes, a few cross section measurements are much superior to a large number of longitudinal section measurements.

B) *Area Measurements:* Measurements of areas were done with a Zeiss integrating eyepiece micrometer. Full details on its theory and operation are contained in reference 12. In brief the method involves the use of a reticule in the eyepiece which casts a series of points upon the image from the objective of the microscope. There are a fixed number of these points — or line intersections — in the reticule. The percentage of intersections overlying the phase to be measured (such as tetracycline labelled bone) in a large number of fields with proper sampling is equivalent to the percentage of the volume of the bone phase that is measured. Precision is increased by increasing the number of sections and fields measured. Another method utilizing a filar micrometer may also be tried.[5,9]

PARAMETERS OF MEASUREMENT

It is necessary to adopt the concept of *absolute bone volume*. This is merely the volume of bone remaining after one has subtracted the volume of the various spaces, these spaces being the osteocyte lacunae, the canaliculae, the vascular channels and the marrow canal. The reason for using the concept of absolute bone volume instead of density has to do with the confusion differences in physical density created. These reasons have been discussed cogently by Robinson.[16]

Bone volume measurements are designated by capital V. Accordingly:

1) V_{vasc} is the directly measured vascular area, while V_{lab} is the directly measured tetracycline-labelled area. Both these areas are also the percentage of the local cortical volume comprising each of the measured phases. Marrow cavity is eliminated by measuring the cortex only.

2) To obtain $\underset{abs}{V_{lab}}$ the V_{lab} must be corrected to the absolute bone volume. In another publication it is noted that the average combined volume of lacunae and canaliculae is about 3%.[5] Accordingly: $\frac{V_{lab} \times 100}{100 - (V_{vasc}+3)} = \underset{abs}{V_{lab}}$ or absolute bone volume labelled.

3) Earlier it was mentioned that the *zone of instantaneous staining* with tetracycline is about 4 microns thick, while the zone creeps centripetally about 0.9 microns per day with the daily progression of mineralization.[6] This means that of the observed width of the tetracycline bands in the bone, part is due to the instantaneous width, and part is due to daily accretion. It is the latter we want to measure. Accordingly another correction factor must be determined. If the number of separate tetracycline dosage epochs is (E), the total number of days of dosage is (D), the zone of instantaneous staining thickness is (T) and the amount of new osteoid mineralized per day is (M), the correction factor is $\frac{MD}{MD + TE} = \frac{0.9\ D}{(0.9D) + (4E)} = K_{l.e.}$

This correction factor is tabulated separately for each case since the values for (M) and (T) may undergo future revision. At present they are the average of a number of cases. It would be preferable if they were individually determined in each case.

4) $\underset{lab}{V_{abs}} \times K_{le} = \underset{abs}{V_{lab,\ cor}}$ or corrected, absolute tetracycline-labelled bone volume. This is converted to the percentage of the bone volume formed in a month by multiplication by $\underset{cor}{\underset{abs}{V_{lab}}} \times \frac{30}{D}$, (D) being, as before, the total number of days of tetracycline dosage.

5) The biological half-life of the bone is obtained in months by dividing the percentage of the bone formed per month, $\underset{cor}{\underset{abs}{V_{Lab}}}$ Mo., into 50. Conversion to years is convenient since the numbers are smaller.

In Table I the measurements and data are listed, while in Table II some of the clinical features of the cases are listed.

DISCUSSION

Since in the absence of rachitic and osteomalacic diseases, as much new bone matrix is produced as is mineralized in a unit of time, the present measurements of the volume of new bone matrix *mineralized* per unit time are equivalent to the volume of new bone matrix *formed* per unit of time. This is one way of measuring osteoblastic activity. The ultimate way of measuring osteoblastic activity would be to add to the present measurements the number of osteoblasts required to form this volume of bone. It will be some time before such a measurement becomes possible.

Since, under normal circumstances, as much old bone is removed as new bone is formed the measurements are also a measure of osteoclastic activity in terms of volume of bone destroyed in unit time.

Some comment about individual cases in Table I is indicated.

Case 3 received therapeutic doses of cortisone continuously for more than a year up until death. The effect was manifested by a complete absence of osteoid seams at death, and a marked retardation in lamellar bone formation. More than half of the labelled bone accretion in this patient was fibrous bone deposited on the periosteal surface of the cortex. In spite of the 6 months' period of continuous administration of tetracycline, not one Haversian system was completely labelled by the drug. There is concrete evidence here, and in the repair of this patient's pathological rib and vertebral fractures, that cortisone exerted a much greater depression on lamellar than on fibrous osteoblastic activity.[7]

Case 4 was a child with Still's disease who had been treated with cortisone continuously for more than a year prior to death. The half-lives of 3+ years in cases 3 and 4 are longer than normal, as comparison with cases 5 and 6 reveals.

Case 14 was a man who received tetracyclines twice, the second time being after the onset of circulatory difficulty in the subsequently amputated lower extremity. Observation of the amount of bone mineralized during each labelling epoch clearly reveals that the circulatory difficulty markedly impeded osteoblastic activity. In this instance the patient acted as his own control — one of the unique possibilities inherent in the measurement of osteoblastic activity by the methods in use in this laboratory.

Comparison of the figures for cases 6, 8, 9, 14, and 15 indicate that osteoblastic activity may vary widely from one bone to the next or may be relatively uniform. Apart from vascular disease little is known about local factors which could cause such marked local differences. There must be such factors, however, since the differences in osteoblastic activity exist.

In a previous paper a gradual increase in osteoblastic activity with age as measured in terms of osteoid seams/mm^3 was presented.[8] It is significant in this respect that the measured values of half-life for the clavicle in a group of patients in their 60's average about 8 years biological half-life. While the number of cases is not sufficient for final conclusions, it is instructive to see that remodelling in this age group is not markedly retarded. In case 21, if allowance is made for the effect of remodelling on the amount of tetracycline labelled bone present at the time of measurement — but labelled 9 years previously — the actual half-life of that bone is about 2 years.

In *cases 15 and 16* osteoblastic activity appeared to be completely depressed as determined by counts of osteoid seams (there were none) and as determined by tetracycline labelling of new bone mineralization (there was none). Again, while the number of cases is not large enough to permit a final conclusion, it is significant that the two methods are consistent in their results.

If an approximate, normal value for clavicular remodelling at age 70 is about 0.1/mm^3 seams, with a half-life of 8 years, then 0.1/mm^3 seams is equivalent to 0.5%

turnover per month, and in general the % volume turnover is about five times the seams/mm³. This figure will very likely require modification and qualification in the light of future knowledge. The figure — more properly speaking, conversion factor for seams to volume — does provide a quantitative basis for the measurements of seams/mm³ previously published.

CONCLUSIONS

The biological half-life of some human bones, measured with the aid of tetracycline labelling, varies from 0.2 years for ribs at age 6 months, to about 8 years for clavicles at age 70. Widely varying values may be found in ribs, clavicles, femurs, tibias and fibulas, the longest half-life being over 100 years.

Exogenous cortisone is observed to suppress lamellar osteoblastic activity pronouncedly, but not to suppress fibrous osteoblastic activity to the same degree.

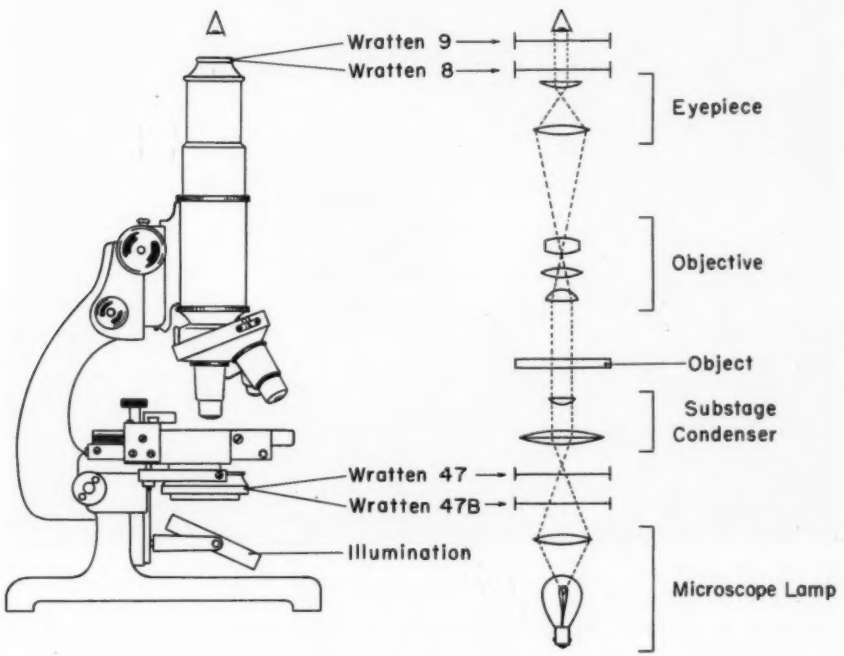


Figure 1

The writer's microfluorescence set-up. The optics are seen in schema on the right. Wratten filters of the numbers listed are used, two in the illuminant beam, two in the beam emerging from the eyepiece. The filters are available as 2-inch gelatin squares from Eastman Kodak Company, Rochester, New York. The illuminant should be a high intensity tungsten source. A 35 mm. slide projector of 150-300 watts will suffice.

A similar set-up, but with different filters, was described in the Austrian literature in the 30's.

Impairment of vascularity is seen to impair lamellar osteoblastic activity.

Correlation between suppression of osteoid seams and suppression of tetracycline measureable bone accretion is observed.

From present data it seems that the percentage of absolute bone volume remodelled per month is on the order of five times the seams/mm^3 measured in the bone in question.

Tetracycline labelled bone has been detected and measured after 109 months of life intervened between labelling and measurement.

By using tetracycline bone labelling in several separate epochs, the organism can serve as its own control in studies of osteoblastic activity.

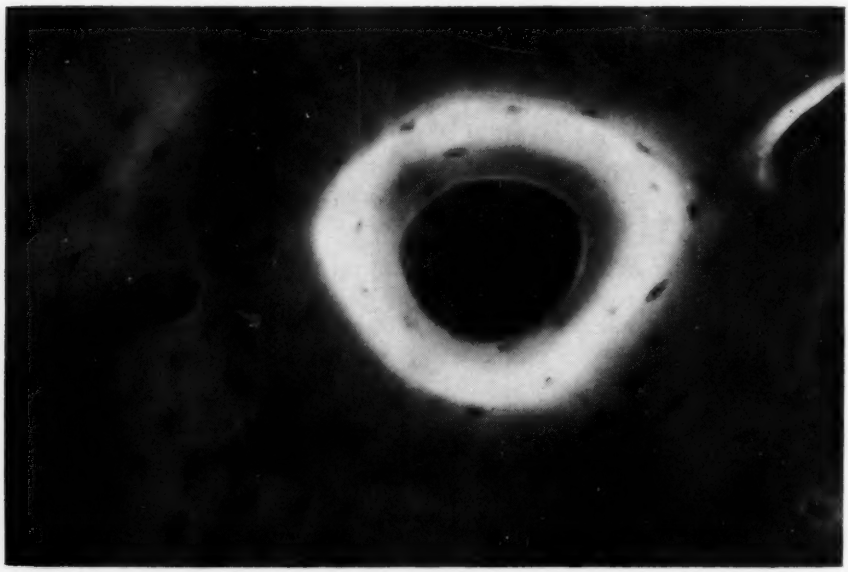


Figure 2

Cross section human clavicle, about 300 x. Writer's microfluorescence set-up.

The bright band is one of the tetracycline antibiotics, administered for 10 days, to a 58 year old man. The Haversian system in the center of this field was being formed during that time. All the matrix mineralizing in those 10 days has been permanently labelled with tetracycline. At 2:00 o'clock another is partly labelled.

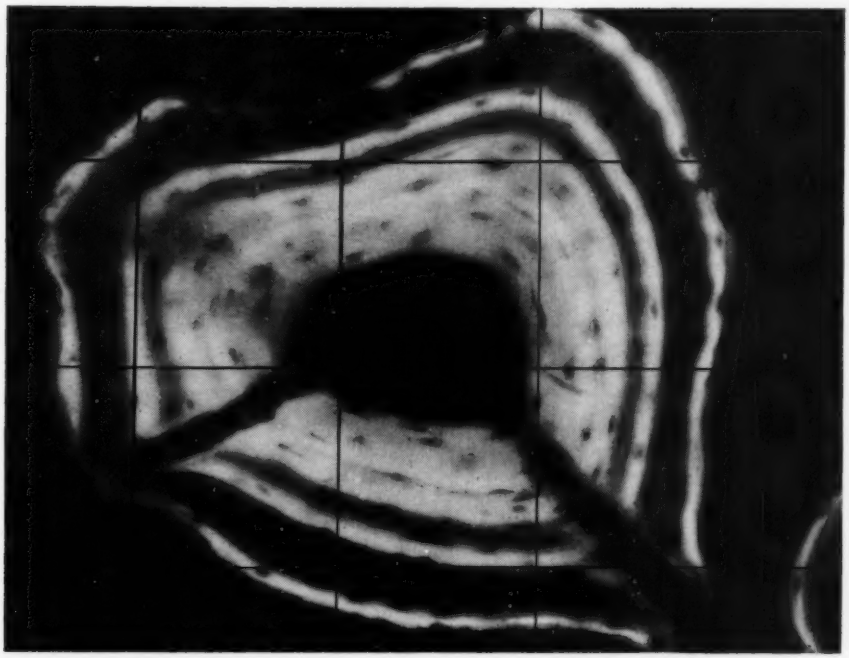


Figure 3

Cross section human femur, about 450 x. Writer's microfluorescence set-up.

A line grid from a net micrometer in the eyepiece is superimposed optically upon the image of the bone section. An Haversian system containing several different tetracycline bands is illustrated. The tetracyclines were given at varying times for a urinary tract infection, the duration of each dosage epoch and separation in days between each epoch being known. Problem: how much of the volume of this Haversian system is labelled with tetracycline, and thus was formed during the time of tetracycline administration?

If each line intersection in the grid is considered a point, and the coincidence with labelled bone of any point a *hit*, then in this photomicrograph there are 12 points, out of which there are 4 hits, or 33% labelled bone. Obviously the accuracy of this one determination is low. Therefore multiple determinations are made by rotating the grid and by moving the section between determinations. The average of all of these measuments is computed. The final figure, for example 18.3%, is the percentage of the area of the microscope field illustrated which contains labelled bone. Because this bone has a longitudinal grain, and is examined in cross section, this figure is equivalent to the percentage of the volume of the bone in the microscope field which is labelled with tetracycline. If the number of days over which the tetracycline was given is known, then it is known from the measurement of area described above how much bone was formed in a known volume of bone in a known period of time.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified sections by rapid manual method, Stain Technol. 33:273, 1958.

2. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

3. Frost, H. M.: Observations on osteoid seams: The existence of a resting state, Henry Ford Hosp. M. Bull. 8:220, 1960.

4. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

5. Frost, H. M.: Measurement of osteocytes per unit volume and volume components of osteocytes and canaliculae in man, Henry Ford Hosp. M. Bull. 8:208, 1960.

6. Frost, H. M.: Lamellar osteoid mineralized per day in man, Henry Ford Hosp. M. Bull. 8:267, 1960.

7. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

8. Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurement with some results, Henry Ford Hosp. M. Bull. 9:76, 1961.

9. Frost, H. M., Villanueva, A. R., and Roth, H.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

10. Frost, H. M., Villanueva, A. R., and Roth, H.: Tetracycline staining of newly forming bone and mineralizing cartilage in vivo, Stain Technol. 35:135, 1960.

11. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:255, 1960.

12. Hennig, A.: Critical survey of volume and surface measurement in microscopy, Zeiss Werkzeitschrift, No. 30, Dec. 1958, p. 78.

13. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

14. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of the tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

15. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

16. Robinson, R. A., Elliott, S. R.: Water content of bone, J. Bone & Joint Surg. 39A:167, 1957.

HUMAN OSTEOBLASTIC ACTIVITY — PART III

THE EFFECT OF CORTISONE ON LAMELLAR OSTEOBLASTIC ACTIVITY

HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

In previous publications we have presented measurements of osteoblastic activity in man. In one series of cases a number of ribs and clavicles were examined and measurements of the number of osteoid seams per unit volume graphed according to age. The curve obtained was a good one and is felt to be highly significant. The material utilized in that study consisted only of patients without known skeletal disease. Exclusions from that study were made, on the basis of hindsight, of patients who were receiving cortisone, ACTH or related compounds for two or more weeks prior to skeletal sampling.

In another publication a true measurement of rate of osteoblastic activity in man was made and expressed as the biological half-life, or the time in years required for new bone formation to reform half the observed, absolute bone volume* if the rate remained steady as measured. Half-life varied from 0.2 to over 100 years. It was possible to deduce that 0.1 osteoid seam/mm³ is approximately equivalent to 0.5% absolute bone volume remodelled per month.

In this paper we present measurements made on a group of patients who were receiving cortisone, a related corticoid, or ACTH continuously for more than 2 weeks prior to skeletal sampling.

MATERIALS AND METHODS

The cases, bones examined, and pertinent vital statistics of the cases are listed in Table I. The methods utilized in preparing the sections, staining and mounting them, measuring them, and the reasons for the validity of the interpretation of the measurements, comprise a bulky lot of publications which will be found in references.[1,2]

OBSERVATIONS

Of the 26 bones from 21 cases in Table I, no active osteoblastic activity was detectable at all in 15 bones and 11 cases. In only two cases, 13 and 19, were measured values of seams/mm³ within normal limits. In the remaining cases a profound suppression of osteoblastic activity had resulted — or accompanied — the treatment with corticoids. Since the reasons for administering corticoids were diverse, it could not be any single disease which produced the suppression. Since the number of cases is rather small, it cannot be reliably concluded that women's osteoblastic activity is more profoundly suppressed by the corticoids than men.

The suppression of osteoblastic activity is apparent when the values listed in the Table I of this paper are compared with the graph in reference 2.

*Absolute bone volume is the skeletal volume remaining after subtraction of lacunar, canalicular, vascular, and marrow space volumes.

Table I

Case	Sex	Age	Bone	Seams/mm³
1	F	13	Clavicle	0.00
2	M	25	Clavicle	0.00
3	F	37	Clavicle	0.00
3	F	37	Rib	0.00
4	M	38	Clavicle	0.017
5	F	41	Clavicle	0.00
6	M	50	Clavicle	0.00
7	M	50	Clavicle	0.00
8	F	51	Rib	0.05
9	M	51	Rib	0.00
10	M	54	Rib	0.00
11	F	54	Rib	0.02
12	M	56	Clavicle	0.034
13	M	58	Rib	0.174
14	M	58	Clavicle	0.006
15	M	58	Clavicle	0.00
15	M	58	Rib	0.00
16	M	60	Clavicle	0.00
16	M	60	Rib	0.00
17	F	61	Clavicle	0.00
18	M	62	Clavicle	0.044
19	M	66	Clavicle	0.03
19	M	66	Rib	0.10
20	F	68	Rib	0.00
20	F	68	Clavicle	0.096
21	M	77	Clavicle	0.00
Mean of entire group				0.022

DISCUSSION

Current theory of factors governing osteoblastic activity states that the protein-catabolic or "S" hormones — cortisones in other words — retard the formation of new bone matrix, and that in conjunction with the fall in output of endogenous protein-anabolic hormone accompanying aging past the 30's, osteoblastic activity is retarded in rate and postmenopausal and senile osteoporoses result. The decrease in endogenous protein-anabolic hormone has been proved. The material presented in this paper proves that cortisone suppresses lamellar osteoblastic activity.

These facts together with the curve of normal osteoblastic activity presented in reference 2, are inconsistent with the protein-anabolic theory of the cause of osteoporosis. If osteoblastic activity actually rises with age, as it has been measured to do under somewhat limited circumstances, then a totally different mechanism must be responsible for osteoporosis than that presently postulated. *The actual mechanism must be an increase in the rate of osteoclasia.* While such an effect has not yet been measured, it has not yet been sought for with the sensitive methods developed in this laboratory.

SUMMARY

Measurements of seams/mm³, by methods previously described, have been made on the bones of 21 patients who were receiving cortisone, related corticoids or ACTH continuously for more than 2 weeks prior to skeletal sampling. A profound depression in lamellar osteoblastic activity accompanied the use of the drug and was almost certainly caused by it.

The invalidation of the theory that the osteoporoses of old age and the postmenopausal state are due to lowered output of endogenous protein-anabolic hormone follows from this and previous work published from this laboratory.

REFERENCES

1. Frost, H. M.: Human osteoblastic activity. II. Measurement of the biological half-life of bones with the aid of tetracyclines, Henry Ford Hosp. M. Bull. 9:87, 1961.

2. Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurement with some results, Henry Ford Hosp. M. Bull. 9:76, 1961.

HUMAN OSTEOBLASTIC ACTIVITY — PART IV

THE EFFECT OF SEVERE CARDIAC FAILURE

HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, R.T.

INTRODUCTION

In previous papers we have outlined methods of measuring diaphyseal osteoblastic activity in terms of osteoid seams/mm³, and given some values in normal and abnormal conditions. In the course of that work the purely fortuitous observation was made that osteoblastic activity appeared to be depressed in many patients who had severe cardiac failure. In this paper we briefly present the data. The bibliography is purposely short. Earlier papers should be referred to by the reader interested in the details.

MATERIALS

We examined 25 bones from 21 patients, the bones being ribs and clavicles. The cases to be included were selected on the basis that they exhibited, during life, two or more of the signs listed in Table I, Columns 6-12. It will help place the present cases, and their results, in perspective if we add that there are 43 cardiac cases in our case material which exhibited only one of the signs in Table I, in Columns 6-12. In these 43 cases there were only two patients with abnormally low values of osteoblastic activity; there was one case in which osteoblastic activity was increased four-fold over the mean.

The low values in the present group of 22 cases stand out much more prominently in the light of the above remarks.

METHODS

The references listed under methods in HUMAN OSTEOBLASTIC ACTIVITY I should be consulted.

DISCUSSION

It is apparent that something accompanying the illnesses of most of the patients listed in Table I markedly depressed osteoblastic activity. It is also apparent that this factor is a permissive rather than necessitative one. By this we mean that the primary physiological disturbance (of cardiac nature) usually, but not always, produces a second, or series of physiological disturbances which retard osteoblastic activity. This is an assumption based on the empirically observed nature of direct and indirect causality in biological systems. In cases of direct causality, X follows Y in 90% — 98% of the observed instances. When X follows Y in over 98% of the observed instances the causality is either inorganic or false. In cases of indirect causality X may follow Y in 10-80% of the observed instances, and this is the present case.

Could the observed effect be the result of an increased elaboration of cortisone-like hormones, coupled with a decreased ability of a liver in a state of acute or chronic congestion to metabolize these hormones? The pronounced depressant effect of cortisone

HFH Case No.	Age	Sex	Clavicle Seams/^{3}	Rib	Leg Edema	Pulm. Edema	Ascutes	Hydrothorax	Enlarged Liver	Fibrillation	Cyanosis	RHD	ASHD	Tetrallogy	Other	Died	Lived	% Change From Expected Normal
20 95 34	39	M	.09	.09	x				x				x			x		0
24 26 23	51	F	.11	—		x			x	x	x	x				x		0
28 35 13	68	M	—	0	x	x		x			x		x			x		100
35 07 46	71	F	.06	.12	x				x	x			x			x		0
39 21 44	57	F	.03	0	x			x	x	x		x				x		—90
40 64 60	38	F	—	.06	x				x	x		x					x	—50
55 98 21	52	F	.11	—	x						x					x		—20
60 16 81	71	F	.12	—	x				x				x			x		0
67 33 14	72	F	—	.08					x	x	x		x			x		—80
88 59 47	51	M	.16	—	x				x				x			x		0
91 32 75	1	M	—	.02					x		x			x		x		—100
91 67 19	60	M	0	—		x			x	x			x			x		—100
92 11 06	38	F	—	0	x					x		x					x	—100
92 28 04	.18	M	—	0	x				x						x		x	—100
92 37 83	.02	M	—	.08	x				x						x			—100
92 40 91	.4	F	—	0					x		x			x		x		—100
92 76 02	59	M	—	0	x	x			x			x				x		—100
93 20 13	45	F	—	.04	x				x			x					x	—80
93 31 82	49	F	—	.06					x	x		x					x	—70
94 56 84	48	F	0	.05	x		x	x	x	x	x	x				x		—75
96 82 06	49	M	.02	0	x	x			x			x				x		—70
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

Table I

The hospital case number, sex, and age of the cases are listed in colums. The bones examined in each case are identified in columns 4 and 5. A dash indicates that the bone listed was not obtained. Columns 6-12 list the major signs noted in the patient's records by the medical staff. It is realized that there may have been some oversights in recording. RHD and ASHD in columns 13 and 14 identify rheumatic and arteriosclerotic heart diseases respectively. Tetrallogy is tetrallogy of Fallot. The two "other" causes of cardiac failure in column 16 were cases with interseptal defects. The cases that lived in column 18 were cases subjected to successful cardiac surgery. In the 43 cases mentioned in the Materials portion of the text there are 21 cases successfully operated on for various cardiac defects.

In column 19 the change from the approximate mean for the age of the patient is listed. The mean is that noted in an earlier paper, "Human Osteoblastic Activity I". The percentage change was computed on the basis that a value of half that normally expected would be valued as minus 50%, while a value a quarter that normally expected would be valued as minus 75%. Values less than 0.1 of that normally expected are arbitrarily valued as minus 100%.

and its relatives on osteoblastic activity previously reported makes such an hypothesis attractive. The hypothesis is quite speculative, however.

It should be noted that the osteoblastic activity referred to in this paper is lamellar osteoblastic activity. Fibrous osteoblastic activity is not considered and it may not be assumed from this work that fracture healing — the important phases of which are the result of fibrous osteoblastic activity — would impaired in patients with severe cardiac failure.

REFERENCES

Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurement with some results, Henry Ford Hosp. M. Bull. 9:76, 1961.

FEATHERING: A THEORY OF GENESIS

HAROLD M. FROST, M.D.

INTRODUCTION

The existence of a new bone affection termed "feathering" and some in vivo characteristics have been described elsewhere.[1] On the basis of the evidence at hand, feathering appears to be a disturbance in mineralization of normal bone matrix. No essential relationship to osteomalacia, to diseases producing osteomalacic changes in the skeleton, or to disturbances in serum chemical values of calcium, phosphorus, acid or alkaline phosphatase have been found to exist in over 300 cases. The organic matrix of feathered bone is normal as determined by ordinary histopathological methods, by the special methods of this laboratory, by phase contrast, polarized light and interference microscopy.

To communicate clearly the physical nature of feathered bone, an analogy will be useful.

Water-Matrix-Mineral Analogy

Assume a glass cylinder (analogous to a unit volume) filled with marbles (analogous to the organic matrix). The spaces between the marbles we fill with water (analogous to the water content of unmineralized bone matrix). Now imagine that finely ground sand is gradually poured into the vessel (the sand is analogous to the crystallites of the bone mineral). The sand grains will fall through the spaces around the marbles to the bottom of the glass. As more and more sand is poured in, two things happen. Water is displaced from the glass so that when all the sand possible has been poured in, there is a minimum amount of water in the vessel (analogous to completely mineralized bone matrix). Second, there is a maximum amount of sand present in the container which is determined by the geometry and size of the marbles and sand grains. As an aside note that the amount of marble in the glass is unchanged as these things occur*, and that the mineral and water content shift inversely.

In feathered bone the organic matrix is normal in amount but there is an abnormally small amount of mineral and an abnormally large amount of water in a unit volume of the matrix. In terms of our analogy, something arrested the pouring in of sand before the spaces between the marbles had become filled with all the sand they could contain.

Decreasing Rate of Mineralization

In normal humans newly formed bone matrix mineralizes rapidly at first and is normally mineralized in 3 months or less after its formation by osteoblasts. The term "normally mineralized" is deceptive. There is no arbitrary end point in the progressive mineralization of bone matrix during life. This is where our analogy falls down. Mineralization actually proceeds at an exponentially decaying rate. The first half of the mineral load a unit volume of matrix can accept is deposited in less than a week, half of the remainder in less than a month, and so on. Even in bone moieties as old as 70 years, a trace of mineral accretion probably occurs each year as long as life

*The reader with a bent for physics will appreciate immediately the value of discussing these events in terms of unit volume instead of in terms of physical density.

continues. Fig. 1.) At present the quantitative aspects of the decay rate and shape of the curve are not known.

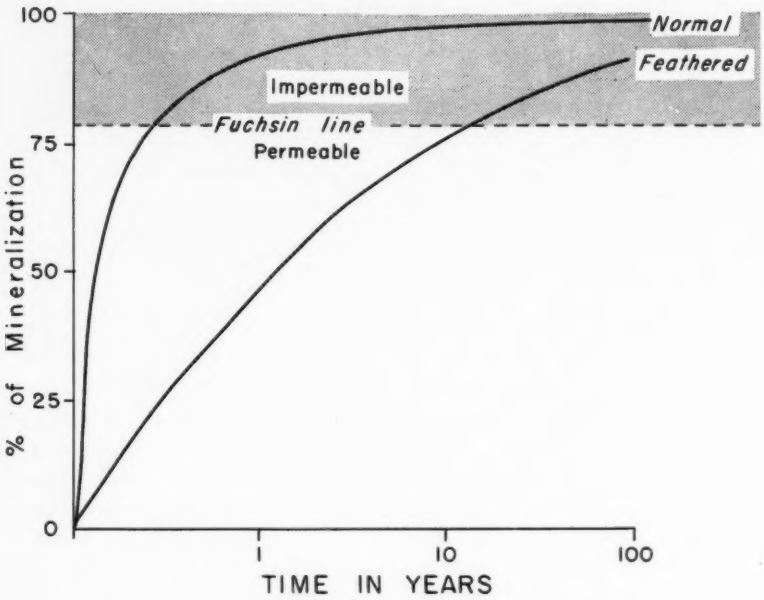


Figure 1

A schematic representation of the relationships between degree of mineralization of bone per unit volume, the time required to achieve a given level of mineralization, and the fuchsin line. Fresh, undecalcified, hydrated, unembedded bone is impermeable to the basic fuchsin standard when mineralized to any degree above the fuchsin line. The same bone is fuchsin permeable, and thus diffusely and deeply red in stained sections properly prepared, when mineralized to a lesser extent than the fuchsin line.

The upper line in the chart depicts the rate of normal mineralization. The lower line depicts what happens to mineralization rate in feathered bone. The time scale is logarithmic, the mineralization scale linear.

It becomes evident that feathered bone must possess some peculiarity in addition to insufficient mineralization, since this alone would not enable it to be distinguished from new bone. The additional peculiarity is the duration for which feathered bone remains fuchsin permeable. This will be explained shortly.

The Fuchsin Permeability Standard

In this laboratory the permeability of fresh, unfixed, undecalcified bone to a 40% ethanolic solution of C.C. basic fuchsin has been adopted as a bone permeability standard. It is a peculiar characteristic of bone mineralized below a certain degree that its partially mineralized matrix is diffusely permeable to this reagent, while bone which is mineralized over this degree is as impermeable as glass to this reagent and takes the stain only on exposed surfaces. The fuchsin reagent thus separates bone into two moieties: that part mineralized above (impearmeable) and that part min-

eralized below (permeable) the fuchsin line. The *fuchsin line* lies roughly at about 80% of the maximum amount of mineral a unit volume of matrix can accept and is the point at which the bone becomes fuchsin impermeable. (See Chart I.)

Fuchsin permeable bone, which does not contain osteoid seams, is microscopically granular with numerical apertures of 0.65 fully illuminated, and which does contain certain specified features at the walls of Haversian canals and at cement lines, is termed feathered bone. The amount of feathered bone in a skeleton may vary from zero to over 75% of the skeletal volume and is more marked in the axial than in the appendicular skeleton. Feathered bone is softer and strains more easily than normally mineralized bone. The theory of genesis will now be developed.

MATERIALS AND METHODS

Sections and Cases

The information on which this paper is based was drawn from the examination of over 4,500 fresh, undecalcified sections made from the bones of over 500 patients of all ages, both sexes and with a wide diversity of pathology. Sections were made and stained, and examined, by methods outlined elsewhere.[2,3]

In vivo Bone Labelling With Tetracycline Antibiotics

Over 90 cases are available in which tetracyclines were given at one or another time prior to skeletal "sampling" (at postmortem or surgery). It has already been recorded that the tetracyclines label newly mineralizing bone in much the same manner that alizarin, radiocalcium or radiophosphorus do; that this label is permanent up to the maximum of 9 years observed between the labelling epoch and skeletal sampling; that the labelled bone can be readily observed; that qualitative and quantitative measurements of considerable precision can be made after allowing for suitable correction factors; and that when recently learned facts of lamellar bone physiology and osteoid seam physiology are accounted for valid interpretations of such measurements may be made.[4]

As examples, this laboratory has been able, with the aid of tetracycline labelled material to: measure the time required for formation of Haversian systems; measure the biological half-life of entire bones; measure the biological half-life of Haversian systems; measure the rate and quantity of bone turnover due to remodelling; measure the effect of certain diseases and hormones on osteoblastic activity in man in vivo.[4,5]

Observation of tetracycline labelled material, and measurements made on it, have provided evidence for the following theory and the observations on which the theory is based.

THEORY OF GENESIS, AND UNDERLYING OBSERVATIONS, OF FEATHERING

Theory: Retardation of Mineralization

In Fig. 1 the normal timetable of mineralization of newly formed bone matrix is depicted by a solid line. The decreasing rate of mineralization with time is diagrammatic. If something were to retard the rate of mineralization of new matrix so

that it required longer to reach the fuchsin line, the result would be something similiar to that depicted by the second line, Fig. 1. Bone moieties which were victimized by retardation of mineralization would thus still be fuchsin permeable at older and older ages in fresh, undecalcified sections. Note that complete arrest of mineralization is not assumed, and that in theory there may be a continuous spectrum of degrees of retardation of mineralization. It is this lag in mineralization, and thus prolongation of the time during which the bone moiety remains below the fuchsin line (fuchsin permeable) that differentiates feathered bone from normal bone dynamically.

Observations:

Numerous examples of feathered Haversian systems labelled with tetracyclines in vivo as long as 9 years before skeletal sampling have been found in the laboratory's material, indicating that feathered bone at least *can* be old. It is characteristic of such old, feathered Haversian systems that considerable differences in mineralization, and thus in fuchsin permeability, occur in the same Haversian system.

Counts of feathered and unfeathered Haversian systems in tetracycline labelled specimens reveal that the shorter the time between the tetracycline labelling epoch and skeletal sampling, the more feathered Haversian systems are found of the same age as the labelling epoch, while the longer between the labelling epoch and sampling, the fewer feathered Haversian systems of the same age as the labelling epoch occur. This is compatible with, and suggests that, feathered bone gradually mineralizes and eventually becomes fuchsin impermeable, but requires considerably longer to do this than is normal.

Theory: Retardation of Mineralization Leads to Accumulation

It is known that even in adult life a constant remodelling of the skeleton occurs, old bone being resorbed and replaced by new. In fact, it is retardation of mineralization of this new bone that creates feathered bone, Normally, as many new moieties of bone are formed in a month as there are older ones mineralizing past the fuchsin line per month. The result is a rather steady state: an average, normal, relatively constant amount of fuchsin permeable bone on cross section of, say, a clavicle.

In the original paper on feathering, it was pointed out that there is no increase in the amount of remodelling in feathered bone, thus no more new bone moieties entering the field per unit time than is normal. Additional observation since that paper was written has amply confirmed the statement.

If this is the case, then marked retardation of mineralization of newly formed bone moieties should lead to a gradual accumulation of larger and larger amounts of fuchsin permeable, feathered bone on cross sections. This is because the bone moieties are not mineralizing past the fuchsin line as fast as they are entering. In metaphor, water is escaping more slowly than it is being poured in, so the amount in the vessel must increase.

Observations

In some bones of some skeletons more than 75% of the bone volume is feathered. The feathering is always demarcated by cement lines, indicating that time, rather than proximity of one bone moiety to another, has something to do with the feathered state.

Theory of Accumulation

The evidence available is consistent with and supports the theory that feathering is caused by somthing that retards mineralization rate some time after the bone matrix is formed but before it has mineralized to the fuchsin line. The theory may be aptly illustrated by another analogy, the water-gelatin-sand analogy. (Fig. 2)

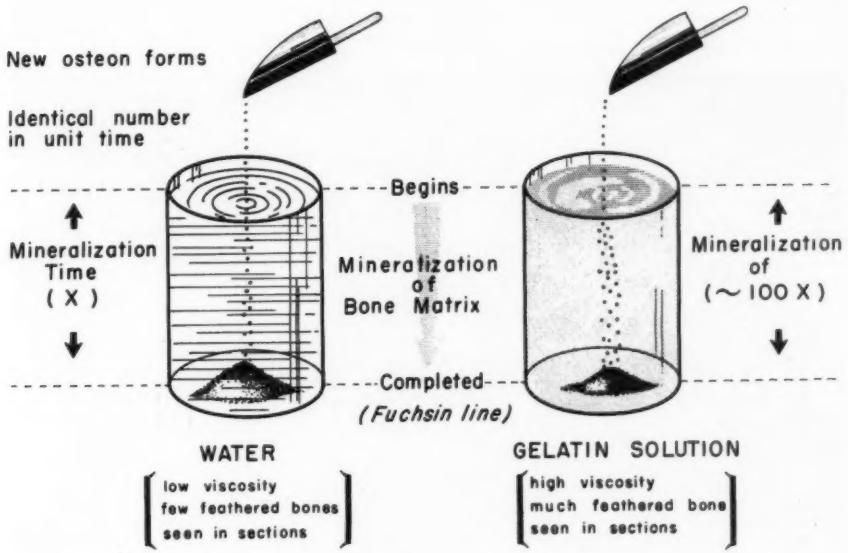


Figure 2

Analogy depicting the difference between the mineralization rate in normal bone (left) and in feathered bone (right). New sand grains (osteons) are introduced at the same rate into each vessel. The individual grains fall from top to bottom of the liquid (mineralize above the fuchsin line) faster in the water (normal) than in the gelatin (feathered). Many more sand grains are in transit at any instant in the gelatin solution due to the retardation of rate of fall resulting from the gelatin's viscosity.

Assume two similar glasses, one filled with water, the other with a gelatin solution. Into each glass drop one grain of sand per second. After a few moments it will be seen that there are many more grains of sand in transit in the gelatin glass than in the water glass, and that it requires longer for a single grain to transit from top to bottom of the gelatin solution than of the water. The analogy is completed by equating the sand grains to new Haversian systems, entry into the liquids as completion of formation of the matrix, transit through the liquids as progressive mineralization, and reaching the bottoms of the glasses as acheiving enough mineralization to pass the fuchsin line. The viscosity of the gelatin solution will determine how many sand grains are in transit through it at any one moment, just as the degree of retardation of mineralization will determine how much of the bone is eventually composed of feathered bone. (Fig. 3A-F)

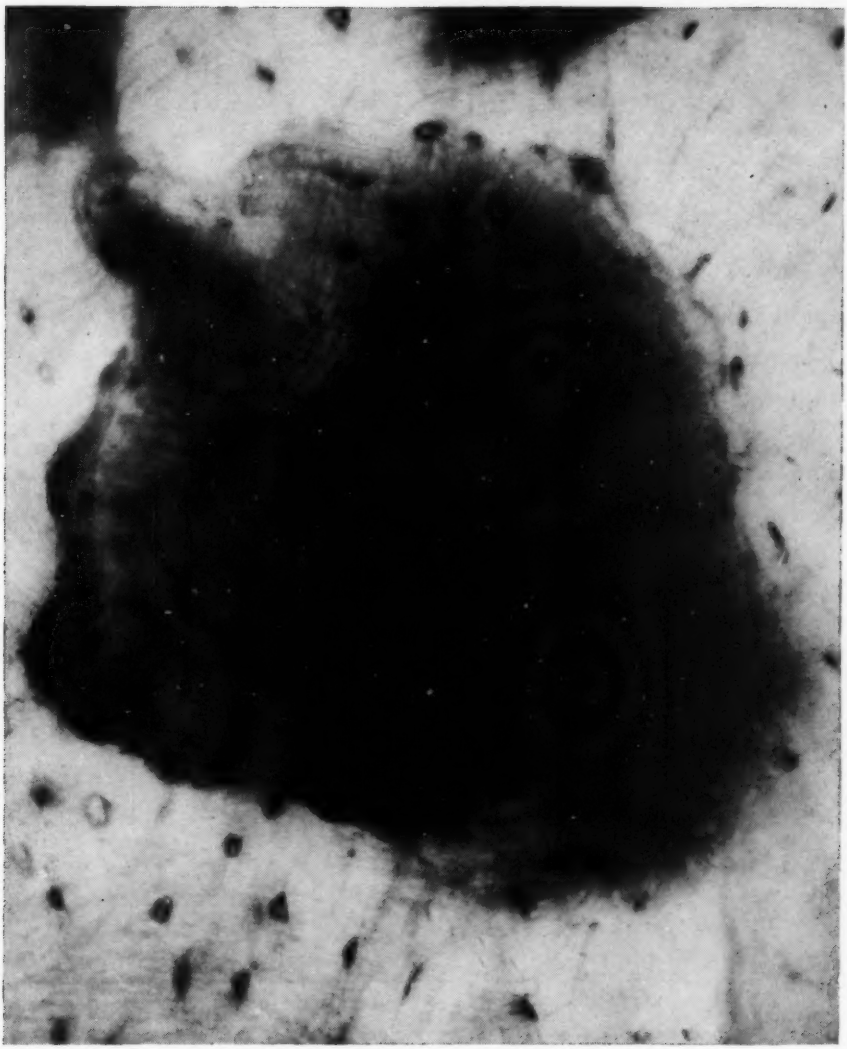
Figure 3A-F

Undecalcified cross sections human clavicle, Frost's fuchsin technique. 200X.

The series illustrates the spectrum of morphological changes that may be seen in a single section. These changes bridge the gap from new-forming Haversian system with an osteoid seam (3A) to a feathered osteon that is nearly completely mineralized past the fuchsin line (3F) diagrammed in Fig. I. The clear areas in the photomicrographs are "normally" mineralized bone which is mineralized above the fuchsin line of Figure 1.

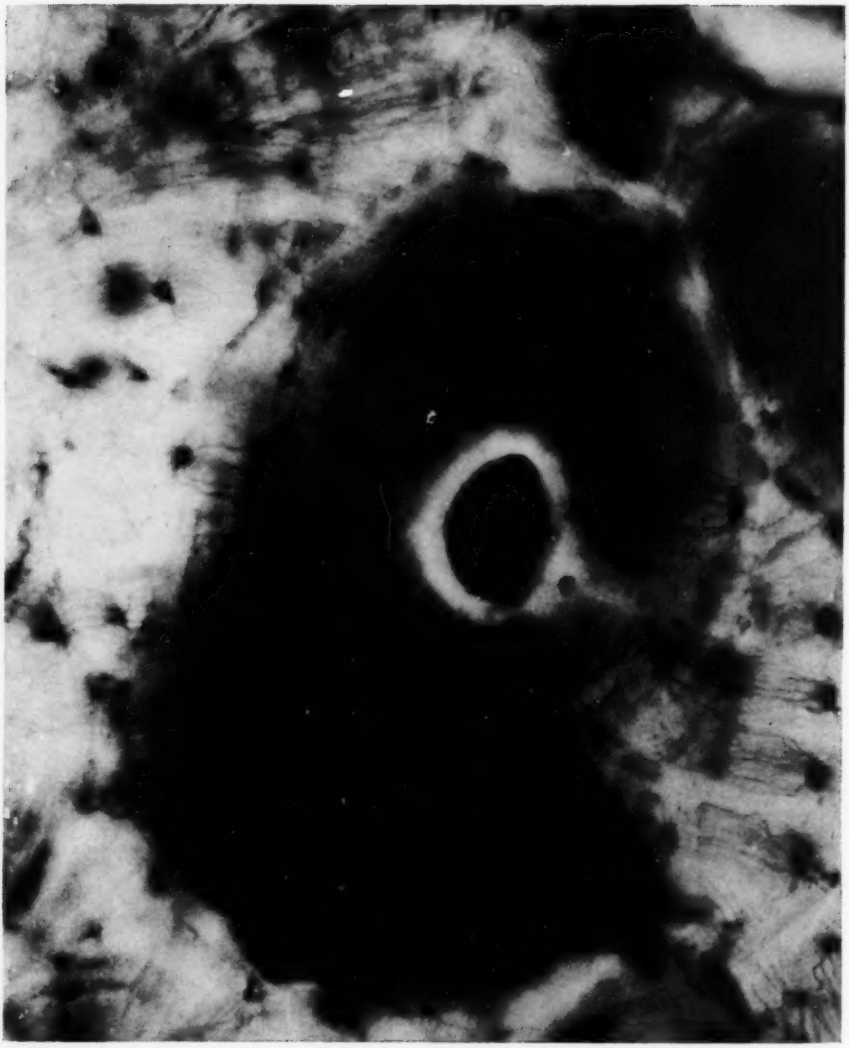
The dark areas in the photomicrographs are mineralized below the fuchsin line, thus are diffusely stained by the fuchsin, and black in the photos by the grace of a Wratten 58 filter.

The central, circular black areas are the Haversian canals.



Theory of Cause of Retardation of Mineralization: Vascular

Two things come to mind as possible causes of mineralization retardation. The first is a humoral factor circulated by the blood which, by exerting an effect on the activity coefficients at the interface between bone crystallites and their solution phase, delays progressive addition of new mineral.

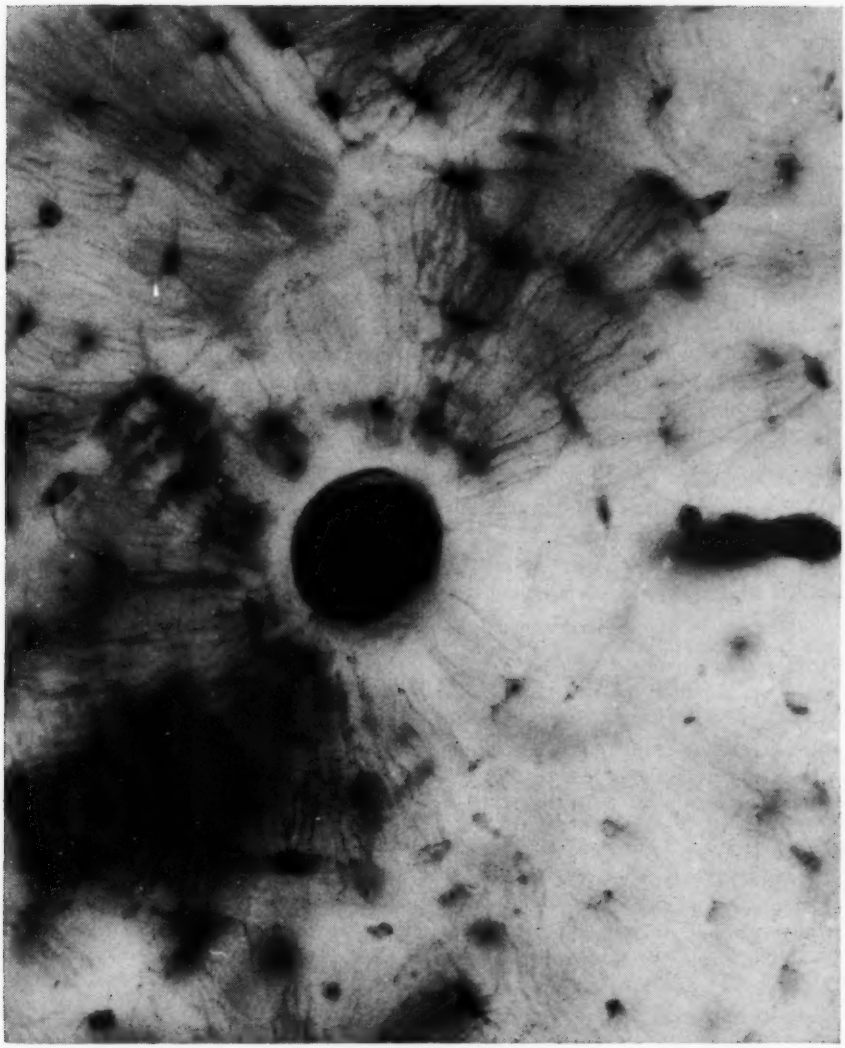


The second possibility is that some vascular event is ultimately responsible for feathering. To check these possibilities, it is only necessary to find out whether the majority of the Haversian systems forming at a given time become feathered, or whether only a few of them become feathered. The reason is the obvious one: a humoral agent should equally affect all mineralizing bone, while the known nature of vascular phenomena is that they are spotty and affect only a fraction of the vessels in a given circulatory bed.

Observations

Examination of tetracycline labelled material clearly reveals that at the most about 30% of the Haversian systems forming during a labelling epoch become feathered, and usually the percentage is lower than 30%. This suggests a local rather than humoral etiological factor, and a vascular event of some sort (thrombosis? embolism? pathological vasospasm? shunt? hemorrhage?) is a likely candidate for a local factor.

In making these observations another thing was noted: an increase in the amount of feathered bone produced during episodes of severe illness. One clavicle is typical. About 40% of the Haversian systems in it were feathered, and of the feathered Haversian systems fully 30% had been in the formative process during a pneumonitis 5 years prior to skeletal sampling and had been labelled by the tetracycline the patient received for a week as treatment for the pneumonitis. Some factor, or combination of factors, accelerated the production of feathered bone by a factor of over 200. The factor must be a potent one and it should be possible to find it if one knew where and what to look for.



DISCUSSION

None of the facts at hand are inconsistent with the theory of genesis of feathering just outlined. The theory is still a theory, however, until direct proof of its details are forthcoming and this should be kept in mind by the interested reader.

It is significant that the existence of a mineralization defect such as feathering was unsuspected prior to the initial report, and it is also significant that previous workers with bone histology and with the physical characteristics, density and ash-matrix ratios did not suspect its existence. In reviewing much of the earlier work in

these fields, one can see fluctuations in the results that were probably caused by feathering. In some instances, particularly in the selection of a method for determination of the ash content of a unit volume of bone, steps were designed into the methods which made them insensitive to the presence of feathered bone.

SUMMARY

Feathering is a newly recognized bone affection. It consists of incomplete mineralization of bone matrix. As far as is known at present, the matrix is normal in feathered bone. Feathering is not an osteomalacic process.

Qualtitative and quantitative observations on the 4,500 + undecalcified sections in the Henry Ford Hospital Orthopaedic Research Laboratory collection of material are compatible with the thesis that feathered bone ooccurs when some agent or agents cause a marked delay in the rate at which newly formed bone matrix mineralizes. As a result of this marked delay and as a result of the fact that there is no change in the rate of remodelling activity in skeletons affected by feathering, feathered bone accumulates in such skeletons. A possible cause of feathering is a vascular event at the capillary level.

REFERENCES

1. Frost, H. M.: A new bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

2. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:272, 1958.

3. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

4. Frost, H. M.: Ouman Osteoblastic Activity II. Measurement of biological half life with some results, Henry Ford Hosp. M. Bull. 9:87, 1961.

5. Frost, H. M., Villanueva, A. R., and Roth, H.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

A POSSIBLE NEW CAUSE FOR ASEPTIC NECROSIS OF THE FEMORAL HEAD

PHILIP H. MCFARLAND, M.D. AND HAROLD M. FROST, M.D.

INTRODUCTION

Aseptic necrosis was first described pathologically by König in 1888 when he labelled osteochondritis dissecans a "quiet necrosis". Since that time we have learned that the blood supply to bone may be interrupted by several known means. These means include physical interruption by fracture, arterial and arteriolar embolism, venous and arterial thrombosis, Caisson's disease, Gaucher's disease, sickle cell anema, hemophilia and others, including the inevitable idiopathic.[1,10,12] We would like to direct attention to the atraumatic causes and present a possible new cause of aseptic necrosis of the femoral head in the human adult.

The currently accepted version of the pathological events that occur in aseptic necrosis of bone divides the phenomena into 3 phases.[1,12]

1) *Onset of cellular necrosis.* This follows interruption of the blood supply, and may be massive or focal, depending on the nature of the vascular disturbance. Death of the cells is followed by autolysis which is apparent microscopically but is not apparent by x-ray. Some time after the necrosis, osteoporosis of the surrounding, healthy bone may begin to develop which leads to a relative increase in radiodensity of the affected bone but not necessarily an absolute increase.

2) *Removal of dead bone.* This stage begins to develop within about a week of the onset of the necrosis and consists of the proliferation of granulation tissue arising from the healthy bone adjoining the dead bone. The granulation tissue absorbs the dead bone with the aid of osteoclasts. This resorptive activity is usually focal, leading to an x-ray appearance in this phase of mottled radiolucencies in the dead bone.

3) *Reossification.* In this phase, regeneration occurs, the areas where dead bone had been absorbed now being filled with new bone, while in other areas new bone is deposited on the surfaces of dead bone trabeculae. Often in this phase there is also some gross crumbling of the affected bone, which in effect, but not in actuality, has been grossly softened by the preceding events. Marked mottling and irregularity appears on the x-ray during this phase because of the osteoporosis of the surrounding live bone, crumbling with trabecular impaction and overlap in the dead bone, focal areas of destruction of old bone, and deposition of new bone on the trabeculae of dead, unabsorbed bone.

In any given case of avascular necrosis of bone, one will probably find all three phases going on simultaneously in different parts of the affected bone, and this contributes materially to the apparent irregularity of the process and the initial appearance of lack of order in microscopic sections.

The authors wish to thank Dr. Richmond Smith, of the Department of Endocrinology, and Dr. J. L. Fleming, of the Department of Orthopedic Surgery, for their contribution of the cases, and for their cogent advice and thinking in the preparation of this paper. The paper was originally prepared for and read before the Detroit Academy of Orthopaedic Surgery in May, 1959, by Dr. McFarland, and with the assistance in preparation of Drs. Smith and Fleming, and is presented here with some modification necessitated by difference in audience and purpose.

We will see a bit later that in some instances this picture of the events is probably too clear and in actuality the events are more complex.

Hypercortisonism: We would like to postulate hypercortisonism as an additional cause of avascular necrosis of the femoral head. It is well known that the pathological changes characteristic of Cushing's disease are simulated by the administration of large amounts of exogenous cortisone. It has come to be accepted that the features of Cushing's disease and of exogenous hypercortisonism are identical. It is known that endogenous and exogenous cortisone have an unfavorable effect on protein metabolism, increasing protein catabolic activity.

It will be recalled that Cushing's disease and Cushing's syndrome are characterized by glycosuria, moon face, buffalo hump, truncal obesity, osteoporosis, thin, reddish skin which bruises easily and contains purplish striae, hypertension, muscular wasting and protruberant abdomen. It is felt that the easy bruising is due to atrophy of the walls of blood vessels.

In recent years we have seen two adults at Henry Ford Hospital who had hypercortisonism and also had avascular necrosis of the femoral head. In one case the source of cortisone was endogenous, in the other exogenous.

CASE PRESENTATION

1) The first case is a 49 year old Caucasian woman who presented with a history of 4 years of increasing pain and stiffness in one hip without any history of trauma. X-rays taken a year before her first Henry Ford Hospital visit revealed an early aseptic necrosis of the right hip.

The woman had the stigmata of Cushing's syndrome by history, by physical examination and on extensive laboratory tests. The cause of the condition was obscure. Although she had been taking cortisone exogenously prior to her first Henry Ford Hospital visit, this was far too short a time to explain her Cushing's picture, the doses were too small, and the x-ray taken a year before her appearance at Henry Ford Hospital antedated the administration of cortisone by more than 9 months. This patient subsequently had a bilateral adrenalectomy. No tumor was found in the adrenals and the patient has been lost to follow-up. The patient's x-rays revealed, in addition to the hip lesion, an osteoporosis.

2) The second case is a 51 year old Caucasian man who developed rheumatoid arthritis 5 years prior to his first Henry Ford Hospital visit, had normal hips by x-ray a year prior to his visit but did reveal osteoporosis on these films, and had been on exogenous cortisone for some time prior to taking these films. When first seen at Henry Ford Hospital in 1957 an early aseptic necrosis of one hip was apparent on new x-rays. A femoral head endoprosthesis was put in this hip the same year, and another one put in the other hip a year later at the insistence of the patient, since that one had become painful in the interim.

When first seen at Henry Ford Hospital the patient had been on cortisone for more than 2 years, had his own source of supply of the drug which he would not

Figure 1

AP x-ray view of both hips in the 2nd case described in the text. The hips reveal changes characteristic of so-called aseptic necrosis, the condition being worse in the hip on the left. Osteoporosis is also apparent.

Figure 2

The hips in the case in figure one after bilateral endoprostheses had been installed by Dr. Fleming.

reveal or discontinue using, and was taking an average of 10 grains of codeine a day. He had the typical moon face, thin skin, purplish striae and multiple ecchymoses when first seen. He would be considered a somewhat uncooperative patient. (See Fig. 1,2,3).

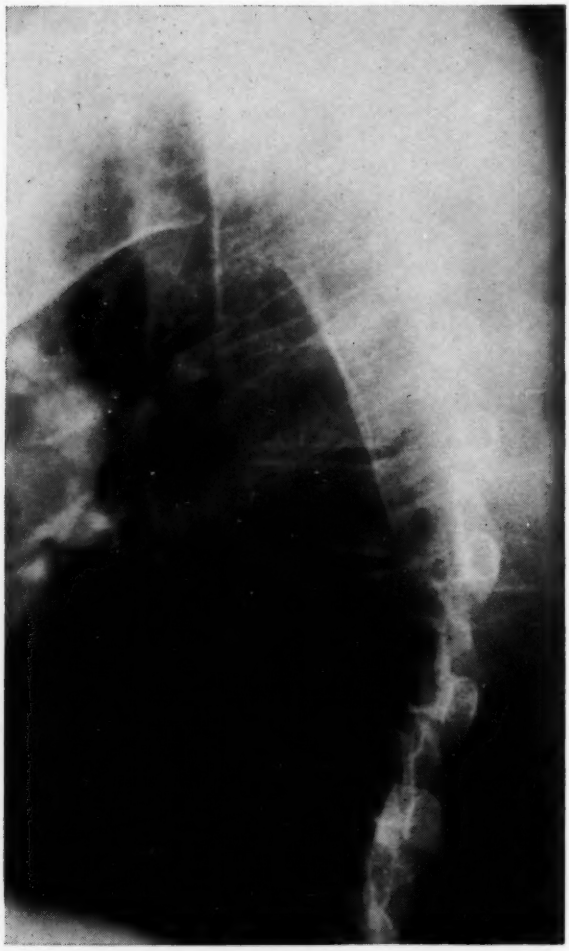


Figure 3

Lateral view of the thoracic spine in the case in figure 1, revealing osteoporosis and somewhat prominent end-plate density.

DISCUSSION

Unfortunately, it was not possible to study the femoral heads in adequate fashion, in the first case, because there was no hip surgery, and in the second case due to error. Consequently proof or disproof of the speculations which follow must await additional material.

Figure 4

Low power photomicrograph of cancellous bone in the femoral head of a patient with rheumatoid arthritis who had received corticoids for a long time. An intracapsular hip fracture led to prosthetic replacement and submission of the specimen for study. The patient had a rather severe osteoporosis. The crosslines identify the area examined under high power in the next figure. Section prepared by Frost's methods.

We may begin with the observation that in two patients who had hypercortisonism aseptic necrosis of the femoral heads developed. In one of the cases the oversupply was of exogenous, in the other of endogenous origin. This correlation has been noted

by others in cases of exogenous hypercortisonism and the presumptive conclusion is that the hormone somehow is responsible for the aseptic necrosis.[11]

How?

We entertain two separate lines of thought.

A) The first line of thought is related to the ease of bruising in hypercortisonism. It is possible that the vascular fragility responsible for this may lead to a focal hemorrhage and avascular necrosis. The femoral head is a likely site for the initial development of such changes in view of the known predilection of the femoral head to develop aseptic necrosis in the presence of the other causative factors referred to at the beginning of this paper.

B) The second line of thought is new and based on work recently done in the Henry Ford Hospital Orthopaedic Research Laboratory.

First, note that the Laboratory has demonstrated the existence of microscopic cracks in vivo in bone.[5] These cracks presumably arise through a process similar to that occurring in the failure of metals.[3,4] A repair process has also been visualized, explaining why these cracks do not accumulate in the skeleton, at least under normal conditions. Some of these cracks have been found to be labelled with tetracyclines administered in vivo, removing the last shred of doubt as to their reality.

Second, many have postulated that cortisone and its related compounds should exhibit a depressing effect on osteoblastic activity in man.[6,7,8] In the past two years the Laboratory has succeeded in measuring this effect in man on lamellar osteoblastic activity by methods described elsewhere. The depressing effect of full therapeutic doses of cortisone and related compounds on lamellar osteoblastic activity may be disconcertingly large. Lamellar osteoblastic activity is reduced from 0.1 to 0.0001 of normal.*

The continual production of microscopic cracks, their continual repair, and the depressing effect of cortisone on osteoblastic activity, may be put together to provide a neat explanation of aseptic necrosis of the femoral head in hypercortisonism. The explanation is so neat that we expect to find some gaps in it later, since biological answers are rarely simple and rarely complete.

The synthesis is simple: We postulate that microcracks are part of the damage normally occurring as the result of daily wear and tear. It is possible that other types of damage also occur which are not as evident as cracks in our undecalcified sections. We know that osteoblastic activity is the only repair process available to bone. It might be expected then that marked retardation of this repair process would lead to progressively larger and larger accumulations of microcracks, and perhaps other types of faults, which in turn lead to the gross crumbling and to the reaction of the adjoining, undamaged tissues which are evident on x-ray and in decalcified sections. This concept also explains the pathological rib and vertebral fractures often found in exogenous hypercortisonism. In figures 5 and 6 appear photomicrographs of actual

*It was also necessary to measure the *normal* osteoblastic activity in man to determine this effect.

Figure 5

High power view of central field in figure 4. A small microcrack is identified by the India Ink lines. This crack lies in the interior of the trabeculum and does not reach the surface of the section or the trabeculae. Plane of focus is about 15 microns beneath the section surface.

microcracks found in the femoral head of a 63 year old woman on exogenous steroids for rheumatoid arthritis. The specimen was obtained at surgery for a prosthetic replacement through the courtesy of Dr. C. White, of the Department of Orthopaedic Surgery.

The nucleus of a new concept is contained in the above paragraph. The idea

is that there is normal skeletal wear, normal skeletal repair of that wear, and interference with either side of the equation may lead to gross pathology. In the present situation we postulate that repair is retarded while wear is not, the result being aseptic necrosis of the femoral head.

It must be noted that our wear-repair hypothesis does not require that the affected femoral head be dead. It may instead be quite alive, at least as far as is consistent with the age of the patient. This is consistent with the findings by Evans and by Frost that there may be disconcertingly little tissue death detectable on sections through a femoral head with aseptic necrosis. It may well be that the term "aseptic necrosis" is merely a convenient clinical description of a symptom-sign-X-ray complex which may have a number of different causes with distinct pathology and pathogenesis. The presence of microcracks and the marked retardation of lamellar bone repair processes which have been found and measured in the Laboratory have not been given consideration in the current theories of pathogenesis of aseptic necrosis because they were unknown when these theories were being formulated.

SUMMARY

Two cases of hypercortisonism accompanied by avascular necrosis of the femoral head are presented. In one case the hypercortisonism was due to endogenous, in the other to exogenous causes. A causal relationship of the hormone to the event is postulated (as it has been postulated by others).[11] Two possible modes of genesis of the femoral head changes are considered, one vascular, the other due to an effect of wear and an effect of cortisone on osteoblastic activity.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases. Philadelphia, Saunders, 1958.

2. Bartter, F. C., Forbes, A. P., and Albright, F.: A comparison of the effect on bone formation of the hyperadrenocorticism of Cushing's syndrome with that induced by adrenocorticotropic hormone (ACTH), J. Clin. Endocrinol. 8:592, 1948.

3. Bechtol, C., Ferguson, A. B., Jr., and Laing, P. G.: Metals and Engineering in Bone and Joint Surgery, Baltimore, Williams and Wilkins Co., 1959.

4. Frankel, J. P.: Principles of the Properties of Materials, New York, McGraw-Hill, 1957.

5. Frost, H. L.: Presence of microscopic cracks in vivo in bone, Henry Ford Hosp. M. Mull. 8:25, 1960.

6. Frost, H. M.: Human Osteoblastic Activity III: Effect of cortisone on lamellar osteoblastic activity, Henry Ford Hosp. M. Bull. 9:97, 1961.

7. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

8. Frost, H. M., Villanueva, A. R., and Roth, H.: Tetracycline staining of newly forming bone and mineralizing cartilage in vivo, Stain Technol. 35:135, 1960.

9. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897-910, 1958.

10. Snapper, I.: Bone Diseases in Medical Practice, New York, Grune & Stratton, 1958.

11. Sweetnam, D. R., Mason, R. M., and Murray, R. O.: Steroid arthropathy of the hip, Brit. M. J. 1:1392-1394, 1960.

12. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.

HALO VOLUME: INTRODUCTION TO QUALITATIVE STUDIES

HAROLD M. FROST, M.D.

The existence of halo volume has been reported previously. In brief, halo volume has been described as a quasipermeable shell of bone surrounding the osteocyte. The shell is the bony wall of the lacuna. The quasipermeability is somehow maintained by the living functions of the osteocyte and disappears after death of the osteocyte.

The significance of halo volume is simple; it is the key to our future understanding of the osteocyte, and it is the locus of rapid exchange processes between bone and blood.

Why study the osteocyte, of all things?

Osteocytes hold the key to a myriad of functions we are just becoming aware of. In a real sense the osteocytes in one's body are a living cellular mass interposed between bone and blood. What exchanges between bone and blood is governed by these cells, and the exchange may involve buffers during severe illness, calcium and phosphate in different types of illness, and toxic bone seeking ions or molecules. To make this gatekeeper do our bidding we must learn its coinage.

One of the trigger mechanisms setting off repair of microcracks is probably osteocytes stimulated in some manner by the cracks. One of the triggers to the normal remodelling of the skeleton which goes on all during life is the osteocyte. Data from this laboratory indicate that changes in the osteocytes are associated with aging and are associated with cardiac failure, particularly fibrillation, the meaning and mechanism of these associations not being known at present.

Ca suffit.

A series of brief papers follow, representing several approaches to the osteocyte problem. It is obvious that we do not fully understand the nature of halo volume, nor the nature of the osteocyte's functions. A great deal of work and thought must be given to the subject before clarity is achieved.

The reader might keep in mind, while reading the following material, the following generalizations:

A) The osteocyte lives in an unusual physical environment consisting of a small hole in dense, impermeable bone. The hole is connected to a source of blood supply by a system of elongated, tortuous tubes of small diameter. The result of this physical environment is that the osteocyte experiences peculiar diffusion problems in getting

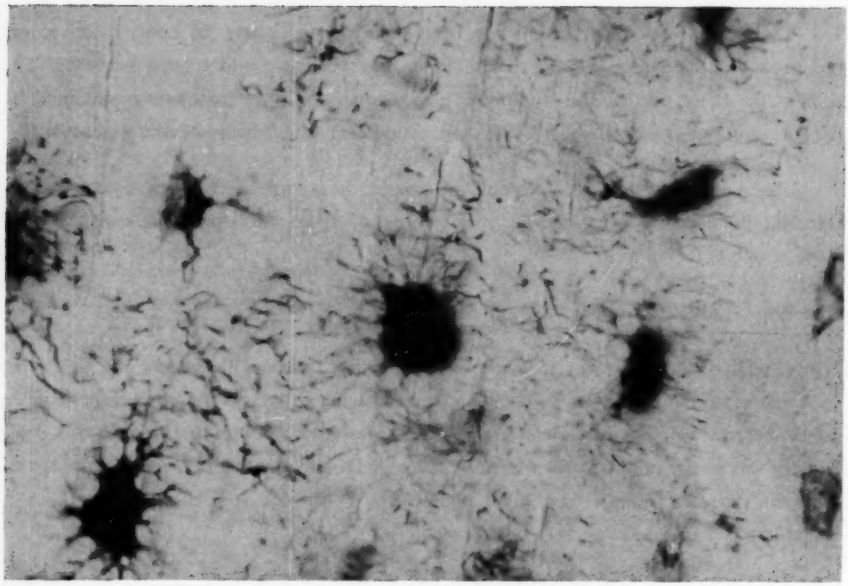


Figure 1

About 600 X, undecalcified human bone, basic fuchsin. This photomicrograph renders a true picture of the relative sizes of the spaces in bone termed osteocyte lacunae and canaliculae. Mineralized bone matrix has prevented permeation of the molecules of basic fuchsin into the matrix. The stain is confined to the lumens of the spaces named, and to the walls of these spaces.

hold of anabolic substances and in getting rid of catabolic substances.

B) The nature of the bony wall of the osteocyte's prison is in turn peculiar. We are just beginning to attack the problem of defining the nature of the bony wall, which we term halo volume. (Fig. 1, 2)

C) The metabolism of the osteocyte is different from that of other cells. Whether this is inherent in the cell or the result of its unusual chemical and physical environment or both remains to be seen.

D) Study of osteocyte metabolism may thus be broken up into study of the nature of the spaces in which the osteocyte resides, the nature of the walls of these spaces, and the nature of the cell biochemistry.

The bibliography follows part IV of this series.

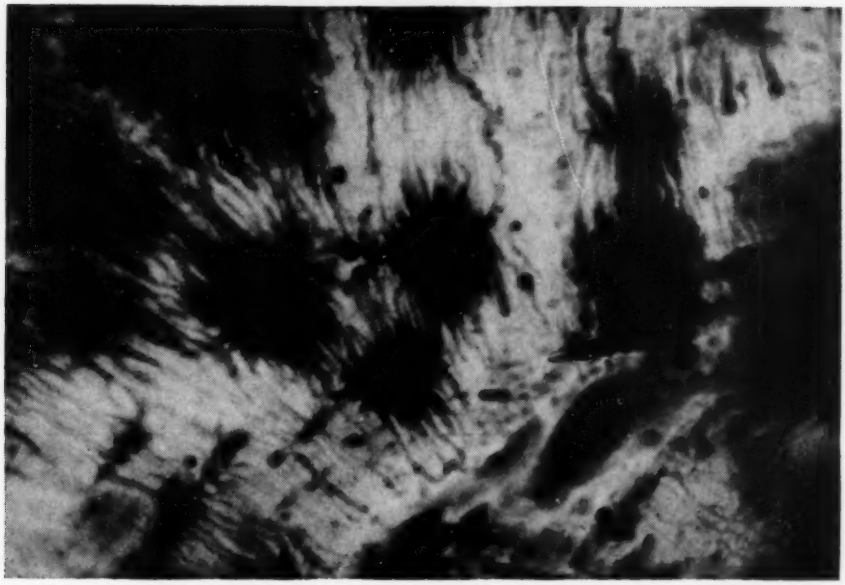


Figure 2

Same magnification, section of same bone but stained for halo volume characteristic by the writer's permanganate method. The lacunae appear fatter, and the parts of the canaliculae close to the lacunae appear larger in diameter, because the permanganate has permeated a short distance *into* the wall of the lacunae and canaliculae. While small ions such as permanganate may permeate in this manner, large molecules such as fuchsin cannot. This selective permeability, in part due to an effect of ionic radius, disappears when the osteocyte in the lacuna dies. The permeability may be decreased or increased by certain observed but not understood effects upon the metabolism of the osteocyte in the lacuna.

HALO VOLUME — PART I

THE EFFECT OF pH ON SIZE

HERBERT ROTH, B.A., HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

Halo volume has been previously described as a semipereable bony shell enveloping osteocyte lacunae and the parts of canaliculae close to the lacunae. It is assumed that the composition of mineral, the state of the mineral, and the exchange between halo volume mineral and blood are governed by some activity of the osteocyte.[11]

Since bone mineral is known to be soluble in acid pH and insoluble in alkaline pH ranges, and since cells may secrete acid metabolites, it seems wise to check the behavior of halo volume to varying pH.

Such an investigation is reported here.

MATERIALS

Five fresh human ribs were obtained from the operating room. The ribs were removed at thoracotomy for varying reasons but not for any bone disease, local or systemic. The ribs were kept moist between the operating room and the laboratory. Since the ribs were obtained on different days, the experiments reported were duplicated with each rib.

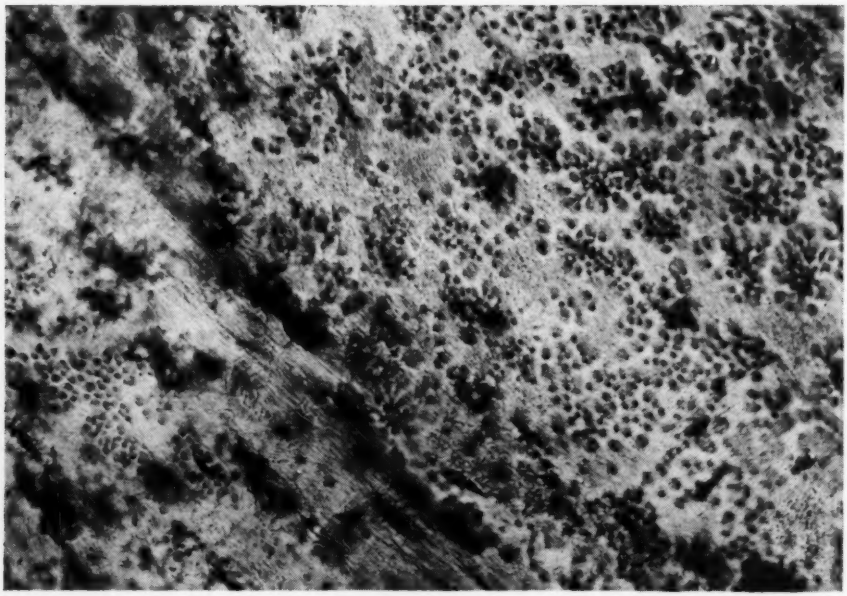


Figure 1

Undecalcified longitudinal section rib. Incubated at pH 8.0 48 hours, then stained with permanganate. The clusters of small, black dots are clusters of canaliculae seen in cross section. Negligible halo around the canaliculae and lacunae.

Halo Volume

METHODS

Fresh, thin, undehydrated, undecalcified, unfixed sections were made by Frost's method.[6] These sections were incubated in test buffer solutions for 48 hours, sections being placed in the solutions within half an hour of removal of the rib from the patient. After 48 hours permanganate halo volume stains were done as described elsewhere,[11] the sections dried and mounted for observation and photography.

Control sections were incubated in distilled water but otherwise similarly treated. Longitudinal sections were prepared from each case. Cross sections prepared in some cases did not yield information not obtainable from the more easily made longitudinal sections.

A Beckman pH meter was used to control pH to the nearest .03 pH units. The pH of the solutions used was 6.5, 7.4, and 8.0.

The desired pH was obtained by mixing suitable proportions of separate solultions of citric acid and sodium citrate. The concentrations of citrate utilized were 0.01, 0.1 and 1.0 molar. For the pH 8.0 solutions small amounts of a 0.1 molar solution of sodium carbonate were added as needed to obtain the necessary pH. Sections were incubated in 30cc. aliquots of the buffer solution at 37 C.

Figure 2

Same material as above but incubated at pH 6.5. Large halo volume around the canaliculae in the center seen in cross section. Some halo volume around some of the lacunae in the bottom third of the figure.

OBSERVATIONS

The criterion of what would be accepted as significant change was an effect obvious to the eye at 100 X magnification. For a number of reasons this is a harsh criterion and change according to it is drastic change.

In sections incubated in the pH 6.5 solutions there were consistently larger and more densely staining halo volumes than in the pH 7.4 specimens. The pH 7.4 specimens in turn displayed larger halo volumes than those incubated in the pH 8.0 solutions and than those incubated in distilled water. See figures 1, 2, and 3.

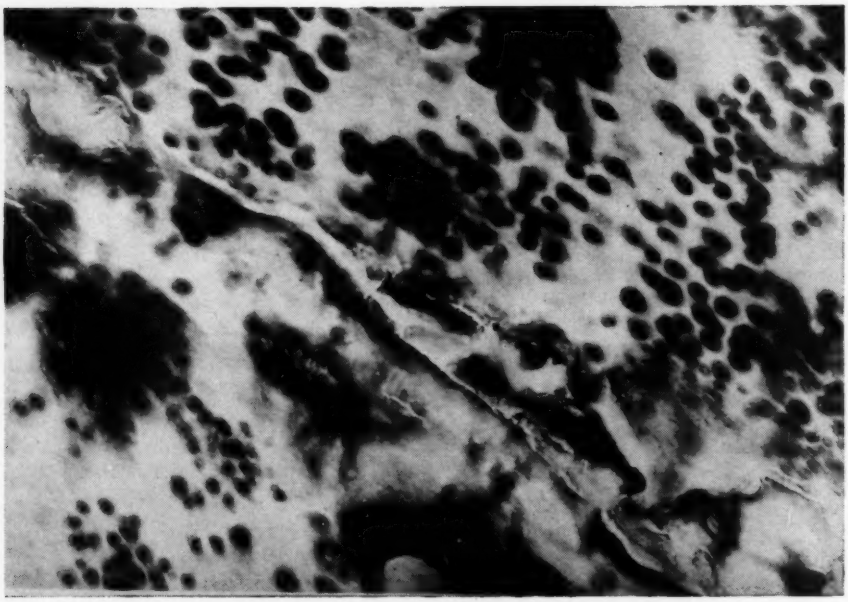


Figure 3

A high power view of section in figure 2. The small, circular, clear area in the middle of the black dots are canalicular lumens. The cylindrical density around the lumen is permanganate-stained bone: the halo volume.

DISCUSSION

Concomitant citrate ion was present in the buffer solution by design. Citrate is probably normally present in vivo.

It may be inferred that in vivo a *possible* means of varying the size and permeability of halo volume would be variation in the acidity of the catabolic products which the osteocyte excretes into the fluids between its cell membrane and the bony wall of the lacuna.

It should be understood that the biologically effective size of the halo volume may be either larger or smaller than the size of halo volumes stained with the recommended methods.

Halo Volume

It may be noted that Frost predicted the existence of enlargement of halo volume in acid pH on simple theoretical grounds,[1] and that Nordin has recently come to the conclusion that such a readily exchangebale fraction of the skeleton must exist in vivo. Nordin's evidence was obtained from tracer dilution and excretion rates with radio calcium and radiostrontium in vivo in humans.[15]

SUMMARY

Halo volumes increase in size in acid citrate solutions.

HALO VOLUME — PART II

THE EFFECT OF CITRATE ION CONCENTRATION ON SIZE

HERBERT ROTH, B.A., HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

Good but not conclusive evidence exists to support the inference that the live osteocytes in bone produce citrate ion in large amounts under certain conditions. If this is true then the citrate ion concentration must be considerably higher within the lacuna than it is in the blood,[2] for reasons discussed later in this series of publications. Accordingly it seems reasonable to determine in vitro what effect varying citrate ion concentration would have on halo volume.

MATERIALS

Five fresh human ribs were obtained from the operating room. The ribs were removed at thoractomy for varying reasons but not for any local or systemic bone disease. The ribs were kept moist between the operating room and the laboratory. Since the ribs were obtained on different days, the experiments reported were duplicated with each rib.

METHODS

Fresh, thin, undehydrated, undecalcified, unfixed sections were made by Frost's method.[6] These sections were incubated in the test solutions for 48 hours, sections being placed within the solutions within half an hour of removal of the rib from the patient. After 48 hours permanganate halo volume stains were done as described elsewhere,[11] the sections dried and mounted for observation and photography.

Control sections were incubated in distilled water but otherwise similarly treated. Longitudinal sections were incubated in each case, cross sections in some cases. No information was obtained from cross sections that was not evident on the more easily prepared longitudinal sections.

A Beckman pH meter was used to control pH to the nearest 0.03 pH units. The pH of the citrate solutions tested was 6.5, 7.4, and 8.0.

Citrate concentrations of 0.01, 0.1, and 1.0 molar were tested. The solutions were made up by mixing separate solutions of citric acid and sodium citrate of the desired molarity. In the pH 8.0 solutions small amounts of dilute carbonate solution were added to obtain the desired pH. Sections were incubated in 30 cc. aliquots of the buffer solution at 37 C.

OBSERVATIONS

The criterion of significant change adopted was a readily evident change in halo volume size and density of stain under 100 x magnification. For a number of reasons this is a harsh criterion and change according to it is drastic change.

In all pH ranges with the exception of pH 8.0, increasing citrate ion concentration resulted in larger size and denser stains of halo volume. See figures 1 and 2.

Figure 1

Longitudinal, undecalcified rib section incubated in 0.01 normal citrate 48 hours at pH 7.4. Small halo volumes. About 80x.

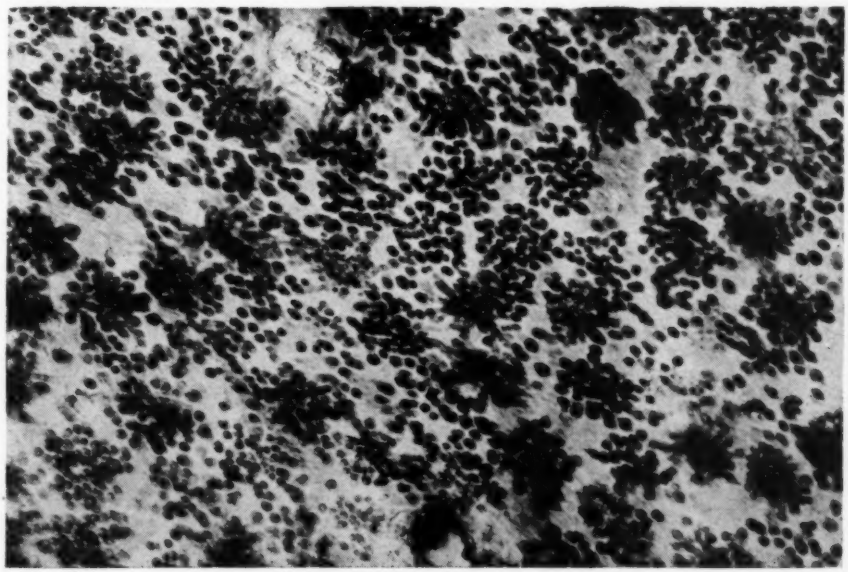


Figure 2

A section incubated in 0.1 normal citrate 48 hours at pH 7.4. Considerable enlargement of the halo volume. The canaliculae are seen in cross section. About 150 x; apologies for not using identical magnifications!

DISCUSSION

It may be inferred that in vivo a *possible* means of varying the size and permeability of halo volume would be by varying the concentration of citrate ion in the fluid between the osteocyte membrane and the bony wall of the lacuna. An abnormally high concentration of citrate ion in the lacuna would result in diffusion of citrate out of the lacunae, through the canaliculae and into the blood. It would appear inevitable that large quantities of citrate produced and secreted into the blood in this manner would carry with the citrate considerable quantities of calcium ion and some magnesium. The reason is the complex formed between citrate and calcium and, to a lesser degree, magnesium.

It is tempting to assume that at least some of the hypercalcemia encountered in hyperparathyroidism and in vitamin D intoxication is the result of calcium removed from the halo volume portion of the skeleton in the manner postulated above. This postulate, while reasonable, is not proved, and other mechanisms beside the citrate — dependent one above, and resorption of bone by osteoclasts, must be sought for.

SUMMARY

Halo volume increases in size with increasing concentration of citrate ion in the lacunar fluid.

HALO VOLUME — PART III

EXISTENCE OF A PATTERN IN THE MATRIX

ANTHONY R. VILLANUEVA, B.S., HAROLD M. FROST, M.D. AND HERBERT ROTH, B.A.

INTRODUCTION

In previous reports concerning the halo volume feature the peculiarities in the mineral fraction of bone have been emphasized. These peculiarities, in brief, consist of a quasimpermeability which is maintained in some fashion only by the living osteocyte. It has been assumed previously that no difference in the organic portion of bone, or matrix, accompanied the difference in the mineral fraction.

In this paper a peculiar halo volume staining pattern present in the matrix under certain conditions is described.

MATERIALS

Perfectly fresh, wet, unfixed ribs from 6 patients have been subjected to the manipulations described next. In addition to this material, various ribs, clavicles, femurs, and tibiae have been subjected to the manipulations referred to, these bones having been kept in bulk in storage in 40% ethanol for periods varying from one to eight months.

METHODS

1) Thin (30 u) undecalcified sections are made. Sections were then washed in distilled water or in 4% formalin; there is no preference for the present purpose.[6] Time: 5 minutes.

2) Sections are decalcified in Versene for one hour. Varying times were experimented with to determine the effect on the final staining pattern. Prolonging Versene decalcification to 24 hours nearly eradicated the halo volume pattern, while 48 hours completely eradicated it. Versene concentration was 1%, pH buffered to 7.0 with sodium carbonate.

3) Sections are again washed in a large volume of distilled water for 5 minutes. The polychrome methylene blue stain used for staining frozen sections in routine clinical pathological work is then applied to the sections for 5 minutes. Sections are again washed in distilled water, 2 changes.

4) Sections are dehydrated in ascending alcohol concentrations, cleared in xylol and mounted in Harleco Synthetic Resin. Sections are handled with tissue forceps during the manipulations described. Sections do not shrink appreciably during the dehydration and clearing. Cross sections, longitudinal sections or both may be used; both were used in the present work.

5) In some instances sections were placed 1 minute in 2% nitric acid after versene decalcification, and then stained. Others, after the exposure to acid just noted, were soaked overnight in 0.1 molar solutions of disodium monohydrogen phosphate, and then stained.

6) In addition, after Versene decalcification some sections were stained with silver nitrate[7] to detect the presence of phosphates and/or carbonates.

7) In some cases, after the recommended Versene decalcification, sections were stained in 0.1% alizarin red S[7] to detect the presence of unremoved calcium.

OBSERVATIONS

A halo volume pattern consistently appears in stained sections decalcified in Versene for an hour. The pattern is faint after 24 hours, and absent after 48 hours in Versene. The pattern is totally absent after a minute's soaking in 2% nitric acid, but is faintly restored in such sections by afterwards soaking for 12 hours in 0.1 molar Na_2 HPO_4.

The silver nitrate stain reveals that after an hour in Versene the only remaining ions which form insoluble compounds with silver ion are in the halo volume portion. Staining with alizarin reveals that after an hour in Versene there is an alizarin demonstrable halo volume pattern left in the sections.

The halo volume stain demonstrated with the above method does not depend on the existence of a demonstrable osteocyte nucleus in the lacuna and is not sensitive to drying, dehydration, and aging as is the mineral halo volume feature reported

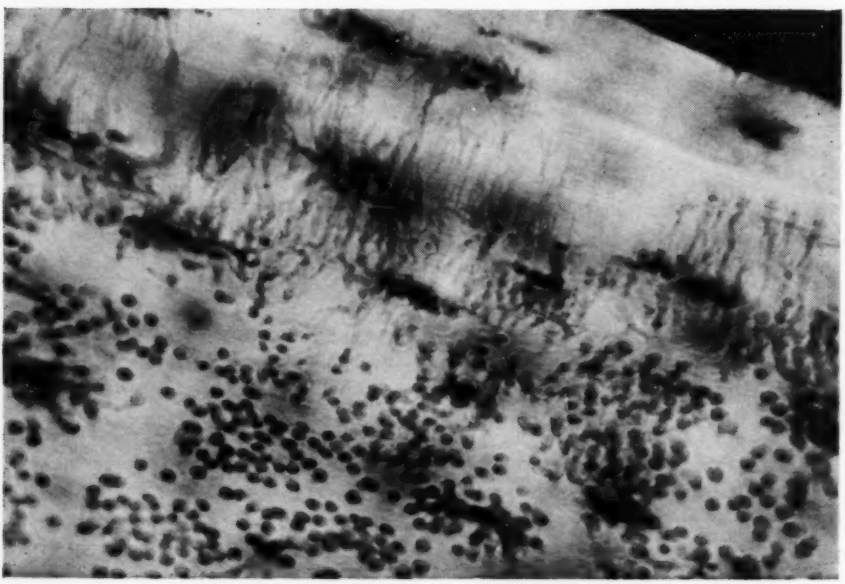


Figure 1

Longitudinal section, decalcified in Versene 1 hour, then stained with polychrome methylene blue. There is more stain present in the halo volume part of the matrix. In the lower half of the figure canaliculae are seen in cross section. In the upper half of the figure some lacunae are seen in longitudinal section. The dark masses in the lacunae are osteocyte nulei.

previously. Storage of bulk bone for up to 8 months did not affect the halo pattern stain visible with the present method. See Figure 1.

DISCUSSION

The consistency with which the halo volume pattern revealed itself with the present technique is proof that it is the result of some difference in the matrix in the stained and unstained parts. The reasoning is simple.

First, there must be some difference in composition of the halo volume part of the sections to account for its consistency denser staining.

Second, the prompt disappearance of this staining pattern in strong acid, its slow disappearance in Versene over longer decalcification times, the presence of silver precipitable ions in the halo volume part of the section and of alizarin demonstrable calcium ions in the same location all mean that the halo volume staining illustrated in Figure 1 is the result of persistent ions in the decalcified matrix. (Recall that Versene may chelate cations in bone but has relatively little effect on anions.)

Third, in order for ions to resist the decalcifying fluid consistently in the halo volume part of the bone there must be " stronger bonds" tying them to the matrix than elsewhere in the bone.

Fourth, stronger bonds mean some difference in the chemistry, sterism or both in the halo volume part of the matrix.

In addition to the above conclusion, several additional things may be deduced from the facts so briefly stated.

First, the chemical peculiarity of the matrix dealt with in this paper is a stable one in comparison to the permeability peculiarity characterizing the mineral halo volume.

Second, being stable, death of the osteocyte residing in the lacuna has little effect upon the existence of a matrix halo volume pattern.

Third, the physical location of the matrix halo volume pattern necessitates the inference that it is caused by some chemical product elaborated by the osteocyte early in its existence.

Fourth, the matrix halo should prove to contain characteristically charged side chain groups.

Fifth, it is necessary to revise our present terminology so that no confusion need occur in the future about which halo volume is being referred to. The following is recommended:

A) Mineral halo volume

B) Matrix halo volume

This terminology is as simple as it is lucid.

SUMMARY

By appropriate methods a halo volume in the matrix of human bone may be demonstrated. Available facts indicate that, in contrast to the mineral halo volume, the matrix halo volume is stable and is demonstrated when mineral ions remain in the matrix halo volume after decalcification but before complete demineralization of the matrix.

HALO VOLUME — PART IV

MEASUREMENT OF THE DIFFUSION PATHWAY BETWEEN OSTEOCYTE LACUNA AND BLOOD

HAROLD M. FROST, M.D.

INTRODUCTION

The osteocyte differs from the rest of the somatic cells in that it resides in a lacuna with walls made of bone. With the exception of halo volume peculiarities,[1] the bone enveloping an osteocyte is impervious to organic and inorganic ions and molecules. If no special provision for diffusion of nutrients existed, osteocytes would promptly die.[2]

A diffusion pathway is provided the osteocytes in the system of canaliculae which connect osteocyte lacunae to vascular channels. This is the pathway through which the average osteocyte obtains anabolic substances and excretes catabolic substances.

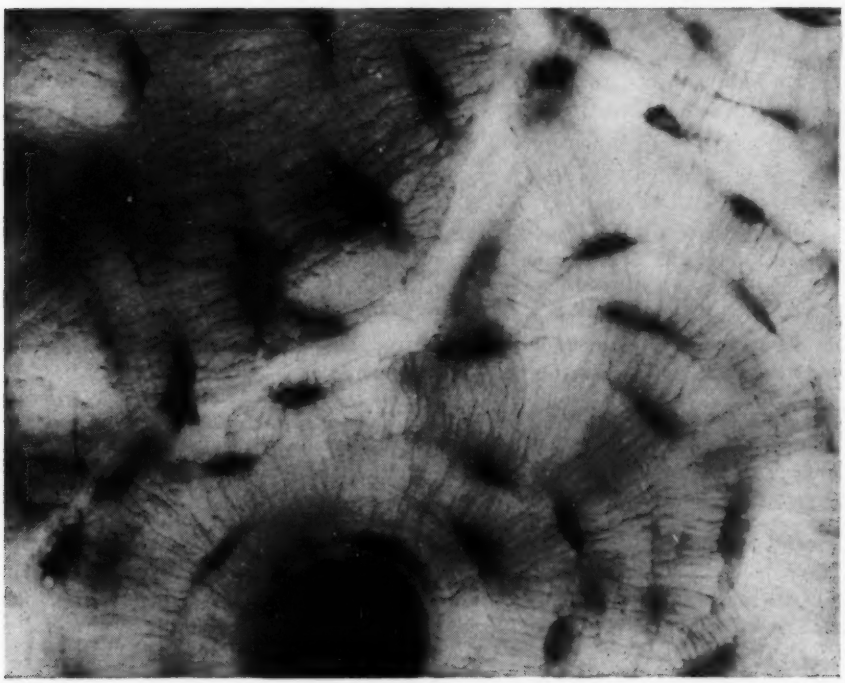


Figure 1

400 x. Fresh, undecalcified, basic fuchsin stained section of human tibia. Cross section.

At 6:30, bottom edge, is an Haversian canal. Numerous osteocyte lacunae are visible. The canaliculae may be seen to run towards the Haversian canal via an interlacunar relay system of anastomoses. A cement line runs diagonally from upper right to lower left. It appears clear because most of the canalicular anastomases normally crossing it are occluded with mineral and so do not stain. This occurs in vivo.

At the upper right corner another cement line is present. Some canalicular anastomoses may be seen bridging the cement line.

The nature, and the effect on diffusion processes, of the canalicular pathway must affect osteocyte metabolism.[1,15]

Canaliculae begin in an osteocyte lacuna. They run from the lacuna through the bone to a vascular channel or, more often, to another lacuna which in turn is connected to a vascular channel through several such relay systems. (Fig. 1). Diffusion impedence is the resistence to diffusion experienced by an ion or molecule. Normally mineralized bone possesses very high diffusion impedence, the canalicular network a workably low diffusion impedence.[2] To understand the diffusion impedence affecting osteocyte diffusion processes, one must know the quantitative anatomy of the diffusion pathway and the chemical, electrical, and solid-state-physical features of this anatomy.

In this paper the quantitative anatomy of the lacunar and canalicular diffusion pathway is described. The data are summarized in part from previously published work and in part from additional work presented for the first time here.

MATERIALS

Fresh, human ribs, clavicles, femurs, tibiae, and fibulas were utilized in the present measurements. The age of the patients varied from 12 through 80, and both sexes were included. No attention was paid to sex or illness in the selection of the material.

METHODS

Fresh, undecalcified, basic-fuchsin-stained sections about 50 microns thin were prepared from the above material by methods published elsewhere.[6,7] The relative lack of shrinkage (0.4% of the longitudinal dimension, 2.2% of the transverse dimensions) is essential for the measurements that have been made. Most of the sections measured were cross sections. Longitudinal sections were utilized for special purposes and for checks on accuracy of certain measurements.

The light microscope was used. Apochromatic objectives, compensating eyepieces, achromatic condenser and full illumination of objectives by Kohler's method were routine. Contrast was increased by using Wratten 58, Wratten 74, or Wratten 45 A filters in the path of the illumination.[14]

Measurements of length (canalicular diameter, distance between vascular channels, lacunar dimensions) were made with a calibrated eyepiece micrometer. Magnification was selected so that the angular width of the individual lines of the scales in the reticule was a minor fraction of the object being measured.[14]

Measurements of volume as percentage of the entire bone volume were made with a Zeiss integrating eyepiece, using a point-counting method described by Hennig, and using the vary shallow depth of focus of an oil immersion objective with N.A. of 1.32 to produce optical sectioning of the bone section.[12] See figure 2.

Measurements of surface area per unit volume were made with a slightly different Zeiss integrating eyepiece which is calibrated against a stage micrometer, has a special reticule and also depends on a point counting system described by Hennig.[12]

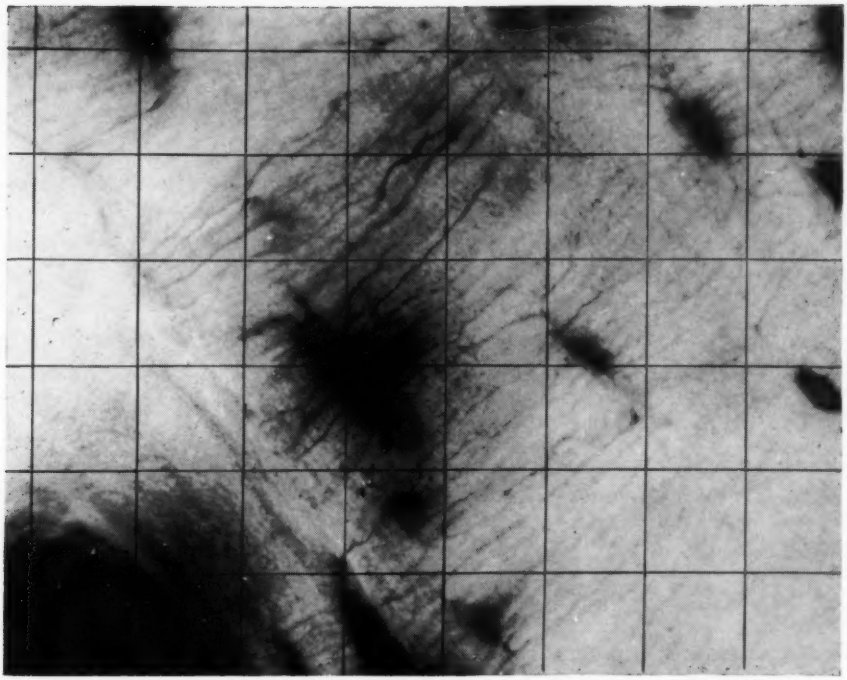


Figure 2

Undecalcified, cross section, human femur, basic fuchsin. 0.95 N. A. The grid overlying this photomicrograph represents the grid of a net reticle in a micrometer eyepiece. The image of the net is superimposed on the image of the bone by the arrangement of optics built into microscopes.

In this net there are 48 line intersections or *points*. If it is wished to measure the percentage of the area occupied by lacunar lumen then each place where a point overlies a lacuna is considered a *hit*. In this instance there is only one hit, to left of center, in a total of 48 possible hits. If a large number of separate fields which are measured in this manner are summed up and averaged, precision increases. The number of fields that should be averaged and the manner of their selection are best determined according to the requirements of the current problem.

In the present instance the depth of focus of the microscope objective is important and is too small for acceptable accuracy, the reason being that it is an appreciable fraction of the dimensions of human osteocyte lacunae. This is one of the many sources of possible error.

When proper attention has been given to sampling technique, to the number of fields measured, to the minimum number of hits acceptable for the desired degree of precision, to the matching of N.A. and magnification to the structure being measured and to the proper relationship of reticle to magnification and N.A.: *then* the measurement of percentage of section area which is osteocyte lacuna (or vascular channels, Haversian systems, canaliculae or whatever) is reasonably accurate.

Since the objective of the microscope is being used to produce an *optical section* through the bone section, an *area measurement* performed along the indicated lines is also a percentage *volume measurement*.

Measurements of lacunae per unit volume was done as previously described.[4]

MEASUREMENTS AND OBSERVATIONS

The data are tabulated in Table I. The *average* values, obtained by lumping values measured on sections from several cases, are listed. The table also lists the estimated probable error of the measurements. The errors are a summation of many

Table I

Measured Value	Number of Cases Measured	Average Value	Approximate Probable Error Plus or Minus	Type of Bone Measured L = lamellar F = fibrous
Osteocytes/mm³	15	20,000	15%	L
Osteocytes/mm³	5	80,000	15%	F
Osteocyte Dimensions Microns	3	4.2 x 11.5 x 24	10%	L
Osteocyte Dimensions Microns	3	7 x 12	10%	F
Osteocyte Volume % of Bone	19	1.5%	10%	L
Osteocyte Volume % of Bone	5	11%	10%	F
Canalicular Diameter Microns	5	0.35	20%	L
Canalicular Diameter Microns	3	0.45	20%	F
Canalicular Volume % of Bone	19	2.2%	80%	L
Canalicular Volume % of Bone	3	1.%	80%	F
Average Separation of Vascular Channels, Microns	12	350	10%	L
Average Surface Area Lacunae, mm²/mm³	5	20	25%	L
Average Surface Area Canaliculae, mm²/mm³	5	90	75%	L

LEGEND: Some measurements from 2 previous papers are combined with additional measurements. Only the averages are given in column 3. Column 2 lists the number of cases averaged. The values in each case were in turn the averages of multiple separate measurements. For example, 150 osteocytes were measured to obtain the average dimensions in lamellar bone; over 300 canaliculae were measured; over 1000 fields were measured in obtaining the volumes of osteocytes and canaliculae.

variables. The measurements with the greatest uncertainty are those made upon canaliculae. The reason is that the average canalicula has a diameter on the order of a wave-length of near-ultraviolet light so that resolving power limitations become serious.

1) *Osteocytes/mm³:* 20,000/mm³ in lamellar bone. Local, random variations occur to the extent of ± 10,000. The average in fibrous bone is 80,000/mm³, variations being ± 40,000.

2) *Canaliculae/mm³:* 750,000 - 2,000,000/mm³. This is a calculated rather than measured value and is based upon the arbitrary definition of a single canalicula as the part of canaliculae lying between one discontinuity and another. Discontinuity here means a lacuna or a vascular channel. The value in fibrous bone is about 20% less.

3) *Canaliculae per lacuna:* 50 in lamellar bone, 30 in fibrous bone.

4) *Canalicular diameter:* 0.35 microns, lamellar bone; 0.45 microns fibrous bone. In lamellar bone canaliculae are oval in cross section, being about 0.4 x 0.3 microns and elongated in the long axis of the bone or Haversian system.

5) *Average lacunar volume:* 310 cubic microns in lamellar bone. 400-900 cubic microns in fibrous bone. There are wide individual variations. Lacunae at the periphery of Haversian systems are consistently larger than lacunae near the wall of the vascular channels. This is a calculated rather than measured volume.

6) *Lacunar wall surface area/ mm^3 of bone:* 20 mm^2/mm^3 in lamellar bone. 80 mm^2/mm^3 in fibrous bone. Variations of half the quoted figures are common. Integrating eyepiece measurement.

7) *Average lacunar dimensions:* 4.2 x 11 x 24 microns lamellar bone. 7 x 12 microns fibrous bone. Lacunar contours are notably irregular, increasing the surface area of the wall with a small increase in volume.

8) *Volume of lacunae as percentage of total bone volume,* vascular volume being accounted for and excluded: 1.5% in lamellar bone. 11% in fibrous bone. Variations of half the quoted values are common. Integrating eyepiece measurement.

9) *Volume of canaliculae as percentage of total bone volume,* vascular volume being accounted for and excluded: 2% in lamellar bone. 1% in fibrous bone. Integrating eyepiece measurements.

10) *Canalicular wall surface area/ mm^3 of bone:* 90 mm^2/mm^3 in lamellar bone. 25mm^2/mm^3 in fibrous bone. Integrating eyepiece measurement.

11) *Average separation of vascular channels:* Measurable on cross sections only, and about 350 microns.

12) *Average distance from average lacuna to vascular channels:* calculated on an area-dependent, rather than volume-dependent basis; 0.33 the average separation of vascular channels, or 115 microns. This is the average length of canalicular pathway along which the substances exchanging between osteocytes and the blood must diffuse.

13) *Diffusion path of average lacuna:* This involves 50 canaliculae of 0.35 micron diameter and 115 microns long. The total tubular canalicular cross section per lacuna is 48 microns.^{2} The total canalicular volume per lacuna is 550 microns.^{3} The total surface area of canalicular wall per lacuna is 6,300 microns,^{2} a proportionately large value which leads to the inference that surface phenomena at the canalicular wall may be important in governing diffusion processes.

DISCUSSION

A) The concept of the average lacuna, the average diffusion pathway, average volume and so on has been introduced deliberately. In order to understand the quantitative behavior of the skeletons' store of osteocytes some quantitative statement of their number and anatomical environment must be made. An anatomical, semi-statistical approach and description should prove useful. In the future it may prove necessary to modify or replace this method of description.

Enough examples have been given to show that conditions present in lamellar and fibrous bone are anatomically quite different.

B) *The average values given above, and calculated below, are for healthy, normal, human, young adults only.* The actual situation in older patients may be considerably different from the present model. The reason is that with advancing age, in man, there is increasing occlusion of anastomoses between the canalicular and lacunar network in Haversian systems on the one hand, and the canalicular and lacunar networks in extra-Haversian bone on the other hand.[9] Such anastomoses create unusually tortuous and lengthy diffusion pathways for the lacunae in extra-Haversian bone and may be the cause, rather than the result, of the notably higher incidence of osteocyte death in extra-Haversian bone as noted by Jaffe and Pomerans,[13] by Sherman and Selakovich,[17] by Rutishauser[16] and by the writer.[8]

In addition to occlusion of canalicular anastomoses at the periphery of Haversian systems, two additional features occur in human bone which may affect the diffusion impedence facing the osteocyte in vivo. Micropetrosis[9] is one of them and feathering[10] the other, the two features producing opposite effects in theory.

The quantitative anatomy of the lacunar diffusion pathway presented is not valid for animals. Enough rodent, dog, cat, monkey, and chicken bone has been observed in this laboratory to make us aware of marked differences in proportions and dimensions of some of the features measured in this paper. The lacunar-canalicular anatomy will have to be measured separately in such animals before meaningful quantitative statements can be made about the chemical behavior of their bone cells or about the surface phenomena affecting blood-bone exchange processes.

C) With the foregoing in mind, some values may be calculated or summarized from the data in Table I which should permit the future design of a quantitative approach to the chemical activity of the osteocyte.

a) *Osteocytes /mm³ of human bone: 20,000.* If a cubic millimeter of bone produces one milligram of citrate per minute, a single osteocyte produces 1/20,000 of this quantity per minute. Not actual values but an example of how the measurement might be used to obtain some basic information.

b) *Diffusion pathway/mm³ of bone:* Since each osteocyte faces average canalicular lumens totalling 48 microns² in area, 550 microns³ in volume, with a surface area of 6,300 microns,² in a cubic millimeter of bone with 20,000 lacunae there are 960,000 microns² cross section area of lumen, 11,000,000 microns³ of lumen with a surface area of 126,000,000 microns.²

Converting the figures to millimeters per mm³ of bone, we have:

c) Canalicular lumen cross section area: 0.96 mm²

d) Canalicular lumen volume: 0.011 mm,³ calculated; 0.022/mm³ measured; 0.016/mm³ mean.

e) Canalicular surface area: 126 mm,² calculated; 90 mm,² measured; 110 mm² mean.

Halo Volume

The agreement between the measured canalicular surface area per unit volume and that calculated on the basis of the number of lacunae per unit volume and the number of canaliculae per lacuna is satisfactory. A similar statement applies to the measured canalicular volume and the calculated canalicular volume per cubic millimeter.

f) Length of canalicular path: 0.115 mm, unchanged from the value for a single lacuna.

g) Lacunar volume: 0.015 $mm.^3$ measured.

h) Lacunar wall surface area: 20 $mm.^2$ measured.

The above figures are for lamellar bone only.[3] Note that for their volume there is an unusually large surface area around the lacunar and canalicular lumens.

D) Note that the *farthest* an ion in the canalicular fluid can get from the canalicular wall is 0.17 microns, but the distance of the *average* ion in the canalicular fluid from the canalicular wall is only about 0.06 microns, or 600 Ängstroms. Half the ions in the canalicular fluids are nearer and half farther than this from the canalicular wall.

These relationships emphasize the earlier speculation that surface effects should prove important in understanding canalicular diffusion processes.

E) The surface through which ions move during exchange between blood and bone is about 130 mm^2/mm^3 of bone, the measured total for lacunae and canaliculae. (The surface area of vascular channels per mm^3 of bone, assuming 10 channels/mm^3 of average diameter of 70 microns, values actually determined by the writer, is only 2 $mm.^2$ an insignificant fraction of the 130 mm^2 surface area supplied by the lacunae and canaliculae.)

It is through the 130 mm^2/mm^3 surface area that tracer doses of radioactive calcium, sodium, phosphate, and strontium move in entering or leaving the skeleton. It is through this surface that lead moves when it enters the blood during an acute acidosis. It is through this surface that radium, polonium, fluorine, and other bone-seeking substances move when depositing in the human skeleton.

F) In the field of physical chemistry it is almost axiomatic that the nature of a surface determines the behavior of that surface. It is probable that a similar effect of the surface composition of lacunae and canaliculae exists and will be observed when sought for.

SUMMARY

The anatomy of the diffusion pathway facing an average individual osteocyte, and facing the sum total of the osteocytes in a cubic millimeter of bone, is described in quantitative terms.

REFERENCES

1. Frost, H. M.: Paper on Biophysical Aspects of Bone, given before the Orthopaedic Research Society, Chicago, Ill., January, 1957.

2. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

3. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

4. Frost, H. M.: Measurement of osteocytes per unit volume and volume components of osteocytes and canaliculae in man, Henry Ford Hosp. M. Bull. 8:208, 1960.

5. Frost, H. M.: Presence of microscopic cracks in vivo in bone, Henry Ford Hosp. M. Bull. 8:25, 1960.

6. Frost, H. M.: Preparation of thin undecalcified bone sections by a rapid manual method, Stain Technol. 33:273, 1958.

7. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

8. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

9. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

10. Frost, H. M.: A new bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

11. Frost, H. M., Villanueva, A. R., and Roth, H.: Halo volume, Henry Ford Hosp. M. Bull. 8:228, 1960.

12. Hennig, A.: Critical survey of volume and surface measurement in microscopy, Zeiss Werkzeitschrift, No. 30, Dec. 1958, p. 78.

13. Jaffe, H. L., and Pomerans, H. M.: Changes in bones of extremities amputated because of arteriovascular disease, Arch. Surg. 29:566, 1934.

14. Needham, G. H.: Practical Use of the Microscope, Including Photomicroscopy, Springfield, C. C. Thomas, 1958.

15. Nordin, D.: Personal Communication.

16. Rutishauser, E., and Majno, G.: Physiopathology of bone tissue: The osteocytes and fundamental substance, Bull. Hosp. Joint Dis. 12:468, 1951.

17. Sherman, M. S. and Selakovich, W. G.: Bone changes in chronic circulatory insufficiency; a histopathological study, J. Bone & Joint Surg. 39A:892, 1957.

HUMAN HAVERSIAN SYSTEM MEASUREMENTS

HAROLD M. FROST, M.D.

INTRODUCTION

An attempt to calculate the physical parameters of the diffusion pathway between osteocytes and Haversian canals created the need for measurements of Haversian system and Haversian canal diameters and their average separation. The majority of such measurements done elsewhere have been on animal rather than human bone. The current measurements are published because they are of some histological interest and because they supply some figures for use in attempting to understand diffusion dynamics in bone's physiological spaces.

METHODS

Fresh, undecalcified sections were made and stained with basic fuchsin by methods published elsewhere.[3,4] Sections prepared by these methods have a shrinkage in the radial and tangential axes of about 2% in adult bone. Accordingly correction for shrinkage is not necessary but if it should be it is readily made. Sections prepared as recommended by Enlow are also satisfactory for this type of work.[2]

Measurements of length were made with a calibrated eyepiece micrometer using fully illuminated apochromatic optics. Magnification was selected so that the majority of the measurements of a given feature involved use of more than half of the micrometer eyepiece scale.[1,4] Orientation of Haversian systems and canals being measured was randomized by arbitrary rotation of the micrometer eyepiece before the measurement and by measurement of structures in all quadrants of the cross section being measured, the exception being the child's rib which did not contain Haversian structures in the pleural cortex. Measurements were made after the line of measurement was made to pass through the center of the Haversian canal by positioning the section for this purpose.

Measurements of area were made with a Zeiss Integrating Eyepiece. This device provides a simple, rapid, and effective means for making area measurements with a microscope to nearly any desired degree of precision and its use is outlined by Hennig.[5]

RESULTS

The measurements are tabulated in Table I.

Note that there is no significant difference in the various measurements with age or sex, in view of the small number of cases measured, and that there is little difference among the various bones measured, the exception being the child's rib.

Note also that the measurements do not reflect the elliptical cross-section geometry of Haversian systems and Haversian canals. These structures are uually ellipses on cross section and the younger the individual the more pronounced is the ellipticity. The measurements listed in Table I are means, due to the randomization of the measurements, and thus are midway between the mean major and mean minor axes of the ellipsoidal configuration of the structures measured.

Table I

Case	Bone	Age	Sex	Average Diameter H.S. u	Average Diameter H.C. u	Average Distance Between H.C. u	Vascular Area In Cortex % of Whole
06 20 70	Clavicle	56	F	258 | 42	57 | 38	346 | 39	6.3
24 30 10	Clavicle	68	F	180 | 15	60 | 12	350 | 19	5.6
38 21 44	Clavicle	57	F	230 | 30	70 | 34	340 | 23	
83 68 47	Femur	53	M	256 | 20	97 | 20	296 | 40	6.15
87 93 01	Clavicle	13	F	300 | 20	69 | 20	385 | 30	12.5
01 18 85	Tibia	90	F	232 | 20	72 | 20	390 | 20	12.4 (a) 39.0 (b)
30 20 60	Tibia	14	M	266 | 20	76 | 30	310 | 20	11.9
94 77 60	Rib	½	F	No H.S.	46 | 20	143 (c) 197 (d)	6.06
Means (94 77 60 excluded)		50		246 | 167	71 | 174	345 | 190	

H.S. = Haversian System.

H.C. = Haversian Canal.

a = outer 1 mm. of cortex.

b = 1 mm band, 1 mm deep to cortical surface (Vascular area in other cases listed is average for entire cross section.)

c = Fetal bone (pleural cortex).

d = Lamellar bone (cutaneous cortex).

The figures in boxes at the lower right of the mean values are the number of measurements averaged to obtain the mean values. Vascular area is the proportion of the total cross section area comprising vascular channels of all types; 11885 excepted as noted in text.

The extremes of the measurements are not tabulated. For the interested reader the minimal diameters of Haversian systems and canals were 30 and 10 micra respectively while the maximal diameters were 550 and 300 micra respectively. The distances between nearest vascular canals on cross sections ranged from 90 to 800 micra.

On the average, in adult cortical bone, the farthest an osteocyte would be from an Haversian canal is half the average distance between Haversian canals, or 172 micra.

Case 11885 is interesting in that this tibia was obtained from a woman with severe osteoporosis. While the outer millimeter of the cortex contained only a slightly increased vascular area, the deeper portions of the cortex contained an increasing area of vascular channel and a decreasing area of bone. This is one of the physical types of osteoporosis, the other being thinning of the cortex without significant enlargement of the vascular channels in the cortex that is present.

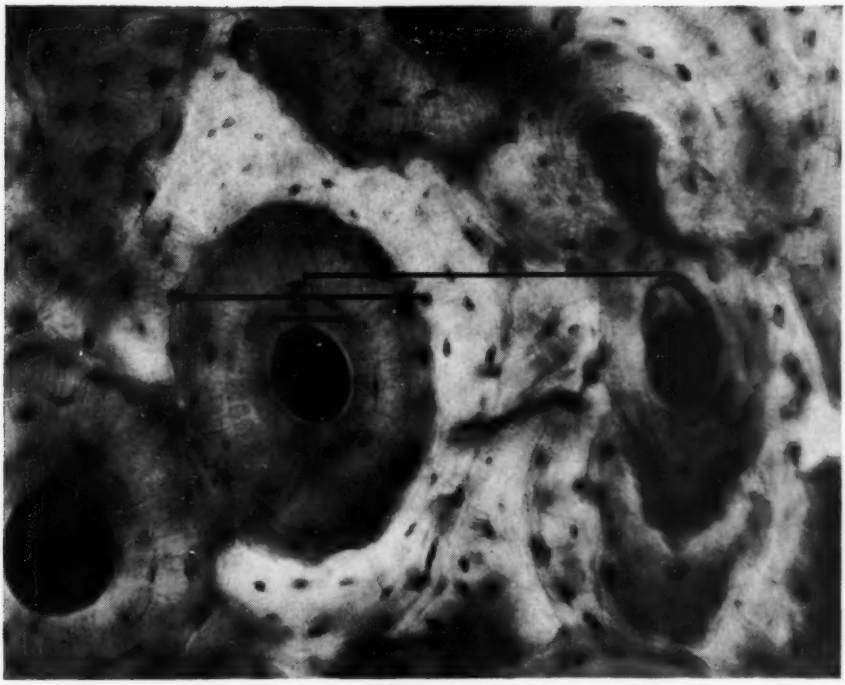


Figure 1

Photomicrograph of cross section human tibia, undecalcified, basic fuchsin. Several Haversian canals and Haversian systems are illustrated. The Haversian system just to left of center is a concentric one, the inner one being the more recently formed. The outer one is larger and appears clear because it is micropetrotic so that most of its canaliculae and some of its lacunae are filled with mineral and unstained.
Diameter of Haversian canal is the feature under the shortest India ink line.
Diameter of the Haversian system is the feature under the middle length India ink line.
Distance between Haversian canals is the feature under the longest India ink line.

SUMMARY

The average diameters of Haversian systems and canals (as measured on 7 human bones) are 246 and 71 micra respectively. The average distance between vascular channels in these same bones is 345 micra.

REFERENCES

1. Allen, R. M.: The Microscope, New York, D. Van Nostrand Co. Inc., 1940.

2. Enlow, D. H.: A plastic-seal method for mounting sections of ground bone, Stain Technol. 29:21, 1954.

3. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

4. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

5. Hennig, A.: Critical survey of volume and surface measurement in microscopy, Zeiss Werkzeitschrift, No. 30, Dec. 1958, p. 78.

6. Needham, G. H.: The Practical Use of the Microscope, Including Photomicroscopy, Springfield, C. C. Thomas, 1958.

PHYSICAL CHARACTERISTICS OF BONE

HAROLD M. FROST, M.D.

INTRODUCTION

The behavior of human, living bone under diverse conditions has long interested, puzzeled, and frustrated investigators. Many examples of peculiar skeletal behavior occur which cannot be understood. Some examples, in brief listing are: recurrence of angulation in greenstick fractures which are not completed; compression failure in some greenstick fractures; bowing of weight bearing long bones in osteomalacic diseases; brittle nature of fractures in aged patients; migration in vivo of many endoprotheses and metallic fixation devices; Otto pelvis; pathological fractures in hypercortisonism; aseptic necrosis of the femoral head; Looser's zones of transformation; march fractures.

A great deal has been learned about the gross characteristics of human bone through study by methods in use in materials engineering. The poor reproducibility of such measurements means that undetected variables are plaguing the investigator. To better understand the whole it therefore becomes necessary to understand the parts of the whole. *This means that attention must be diverted for the time being from the macroscopic and turned to the microscopic.*

In studying the microscopic behavior of bone under load it becomes necessary to combine the methods and knowledge of diverse disciplines. These disciplines include metallurgy, solid state physics, chemistry, structural engineering, materials engineering, histology and physiology. It is also necessary to keep in mind during such studies that the biological side of bone affects the structural side and vice versa. This mutual interaction complicates the problem considerably. Until the attack on the problem has dug deeply enough it is inevitable that mistakes in interpretation of data and in design of experiments will occur. This is part of the growth in knowledge and must be cheerfully accepted.

The following brief series of papers dealing with the physical characteristics of bone are more tantalizing than informative. The observed results raise many more questions than they answer — an indication that the road ahead is a long one.

The bibliography follows Part IV of this series.

PHYSICAL CHARACTERISTICS OF BONE — PART I

THE EXISTENCE OF PLASTIC FLOW IN VITRO

HERBERT ROTH, B.A., HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

Elastic behavior and plastic flow are terms used by metallurgists. When a slightly bent steel wire returns to its original shape and dimensions upon release of the bending force it is exhibiting elastic behavior. If the wire is bent too much it will retain some of the bend. In the region of the wire where the kink occurs there has been a sliding of ultramicroscopic planes of crystals, phase boundaries and dislocations. This sliding is termed plastic flow.

Most solids, glass being an exception, have a crystalline structure. The crystals are of microscopic or ultramicroscopic dimensions and are physically oriented to varying degree with respect to their neighbors. In some materials, such as table salt, the orientation is highly regular, while in others, such as cast iron, the orientation is highly irregular. The difference may be illustrated by the analogy of the disorder present in a pile of bricks dumped on the ground, and the order present after a mason has constructed a fireplace out of them.[2,10]

The various crystals, phases or domains in a structural material adhere to one another by the action of surface bonds of several types. All of these bonds are basically electrical in nature. Springing of these bonds occurs during elastic behavior. Rupture of the bonds occurs during plastic flow, the ruptured bonds reattaching at new sites during the plastic flow.

When plastic flow has occurred a permanent deformation of the material results. This permanent deformation is termed a permanent set.

The actual events during elastic deformation and plastic flow in various materials are usually complex due to microscopic and ultramicroscopic degrees of disorder, differences in composition, corrosion, and other factors. To understand the microscopic events in a material under load it has been necessary to develop and apply analytical methods which reveal microscopic and ultramicroscopic phenomena.[2,5,6,10,11]

Simple experiments are now described which demonstrate that plastic flow may occur in fresh, wet, human bone.

MATERIALS

Two tibias and two femurs were obtained fresh from the operating room, the amputations being done for soft tissue neoplasm in one case and vascular insufficiency in the other. The specimens were kept moist before and during the experiments with tap water applied by suitable means. The patient's ages were 18 and 63 years.

METHODS

Test samples were sawed from the diaphyseal cortices with a fine toothed hack saw and ground with surfaces flat and parallel under running water.[7] Finished samples

were about 10 x 5 x 5 mm and cut so that any of the major axes of the diaphysis were parallel to one of the sample faces. Loads could thus be applied parallel to the longitudinal, radial, and tangential axes.

Test devices were utilized to apply the loads. There were two types.

A) A ram and anvil were machined from mild steel, the ram being a key 3 mm. in width, the anvil containing a slot for the key 0.1 mm. larger in width than the key. (Fig. 1). Ram and anvil were positioned in a vise in such manner that they fitted together when the jaws were closed. A specimen placed between the jaws is squeezed between them. Shear load was applied in the plane connecting the homologous sides of the anvil and ram.

B) The second device was simpler. A dissecting needle was laid with its long axis on the surface of the test sample and the vise jaws closed, squeezing the needle into the substance of the test sample. (Fig. 2). The forces developed in the bone in contact with the needle were complex.

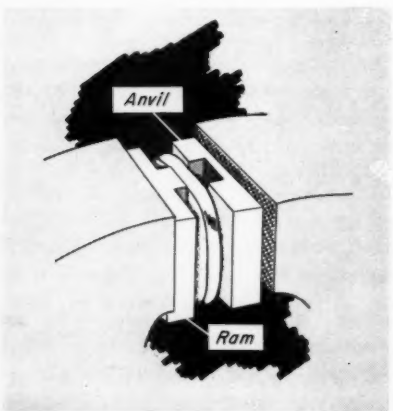


Figure 1

The arrangement of the vise, ram, test specimen, and anvil are illustrated. The ram is a key machined into a block of steel. The anvil contains a slot into which the ram fits.

In use the ram and anvil were glued to the vise jaws with an adhesive. Test samples were then clamped between them and kept moist either with a medicine dropper or with a wick connected to a large reservoir of water.

The plane of idealized shear is parallel to the sides of the ram and anvil and connects the sides through the substance of the test sample.

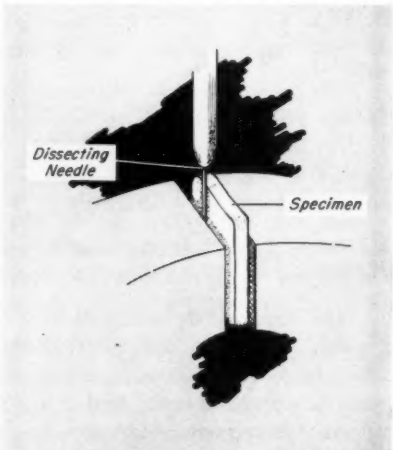


Figure 2

The arrangement of the vise, ram, test specimen and anvil when a dissecting needle is used as a ram is illustrated. The stresses produced in the test specimen in this set-up are complex and no effort is made to describe them.

The test samples were kept moist with tap water before and during the experiments. After the experiments some samples were dried and mounted intact on microslides. In other samples thin, undecalcified sections were made in such a manner that the

surface indentation in the original test sample lay along one of the edges of the finished sections. The sections were then stained with basic fuchsin by methods described elsewhere.[8]

OBSERVATIONS

A) Quantitative measurement of the forces applied is not possible with the present experimental design.

B) Test samples loaded lightly would visibly indent in front of the two rams. Upon removal of the load the indentation completely disappeared, leaving perfectly flat surfaces with no visible evidence of damage.

C) Test samples heavily loaded developed larger indentations under load. Upon removal of the load some of the indentation remained in the surfaces of the test samples. The shape of the indentations was a mirror image of the shape of the rams. (Fig. 3).

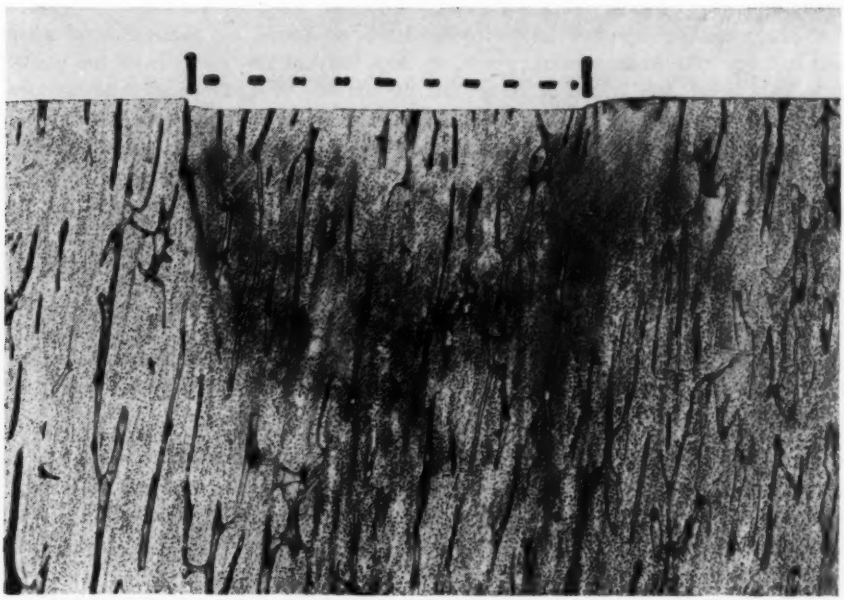


Figure 3

26x. Photomicrograph of section cut through a test specimen given a permanent set by the ram in figure 1. An india ink line delineates the indentation representing the permanent set. Plastic flow had to occur to permit this permanent set.

D) Test samples loaded too heavily fractured in the vise. In the case of the device in Figure 1, the failure was grossly a shear failure, a rectangular piece of the sample being fractured and forced into the slot in the anvil. In the case of the device in Figure 2, the failure was a tension failure, the needle acting as a wedge

which forced the sample material apart in opposite directions. The amount of load increase required to fracture the sample over the amount of load required to produce a permanent set was small in comparison to the total load range within which the material remained intact and did not fracture.

E) Permanent sets could be given to samples loaded in any direction.

Figure 3 is a photomicrograph of a section cut through the surface of a test sample given a permanent set by the device in figure 1. The mirror image of the ram appears along the upper edge of the section as an indentation.

F) In some of the tests samples were left clamped between anvil and ram for prolonged periods of time with loads insufficient to break them or to produce a permanent set within the few minutes required for the experiments described above. The prolonged loading elicited some unusual effect from the sample because often, after many hours, they would suddenly fracture with a readily appreciated loud snap.

DISCUSSION

The preceding experimental description leaves no doubt that plastic flow of some sort can occur in fresh, human, wet bone. A permanent set results from the plastic flow. In a rough way it can be stated that the amount of load necessary to produce plastic flow in short time periods is so close to the amount of load needed to produce failure that plastic flow is probably not normally a skeletal physiological problem. The large range of elastic behavior underlying the point at which plastic flow occurs probably encompasses normal daily skeletal loads.

In children's bones sustaining fractures a permanent set as the result of plastic flow would be even more likely to occur than in adult bone. This is because children's bones contain less mineral per unit volume than adult bone and are accordingly more flexible. The recurrence of angulation in greenstick fractures which are not completed could be explained by the presence of a permanent set. Such a permanent set could be "unset" as a rule only by completing the fracture.

The hours-delayed failure of specimens clamped in the vise for prolonged periods suggests two other factors familiar to metallurgists: creep and fatigue. Creep is a gradual yielding of a structural material under load, such as the sag that develops in the girders of a bridge after 30 years of service. Fatigue is a failure resulting from normal loads rather than excessive loads, and is a complex topic. Additional work will be needed to follow up these suggestions.

SUMMARY

Plastic flow and a permanent set can be produced in fresh, wet, human bone.

PHYSICAL CHARACTERISTICS OF BONE — PART II

BIPHASIC ELASTIC BEHAVIOR OF FRESH HUMAN BONE

HERBERT ROTH, B.A., HAROLD M. FROST, M.D. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

Elastic behavior is the ability of a material to deform under a load, and to resume its normal shape and dimensions after removal of the load. All structural materials are elastic to some degree, including chalk, concrete, and glass.

The basic theorem of elastic theory is Hook's law which states that the strain (strain in engineering is deformation) is proportional to the load. This means that in theory 2 grams load produce twice as much deformation as one gram and that as much energy is returned upon load removal as is put in upon loading.[2,5,6]

No *real* structural materials obey Hook's law perfectly and some behave in very poor elastic fashion. In order to understand the peculiar behavior seen by means of very accurate measurements done over considerable time periods, it has been found necessary to assume that all structural materials contain two separate, differently behaving parts.

1) Purely elastic component. As indicated this component obeys Hook's law and behaves in purely elastic fashion.

2) Anelastic part. This part behaves in totally nonelastic fashion. It is as though fluid were present in a cylinder containing a small orifice. A pull on the cylinder is resisted, and the stronger the pull the stronger the resistence. Due to leakage of fluid through the orifice the cylinder gives. When the pull is removed, the system comes to rest where it happens to be with no tendency to return to its original shape or dimensions. This component resists the correction of a deformation as much as it resists the original deformation. The work put into moving this system is lost as heat or some other form of energy.[2,5,6]

Figure 1 reveals the so-called Zener model of a linear elastic solid. This model provides a good approximation of elastic behavior. Certain discrepancies indicate that the total picture yet remains to be recognized.

Some anelastic component exists even in the very best springs. The result is that vibrations spontaneously die out, even in vacuum. In many real structural materials the elastic behavior is very complex due to varying composition, phase states, and the effects of varying temperatures, densities of intercrystalline bonds, varying velocity of displacement and so on.[2,5,6,10,11] The result of these varied factors is *anomalous behavior*.

While anomalous behavior may be frustrating under some circumstances, it is gratifying when one wishes to learn something about the basic nature of the material exhibiting it. The reason is that the anomalies are the key to the structural characteristics of the material.

The results of simple experiments on the elastic behavior of bone are now presented. The experiments followed a fortuitous observation, reveal anomalous elasti-

city and lead to an unusual inference about the elastic behavior of bone, the inference being that the elasticity in bone is the result of two phases acting in concert within normal load ranges but separated by differing characteristics near failure.

MATERIALS

Two tibiae and two femurs were obtained fresh from the operating room, the amputations being done for soft tissue neoplasm in one case and vascular insufficiency in the other. The specimens were kept moist before and during the experiments with tap water applied by suitable means. The patient's ages were 18 and 63 years.

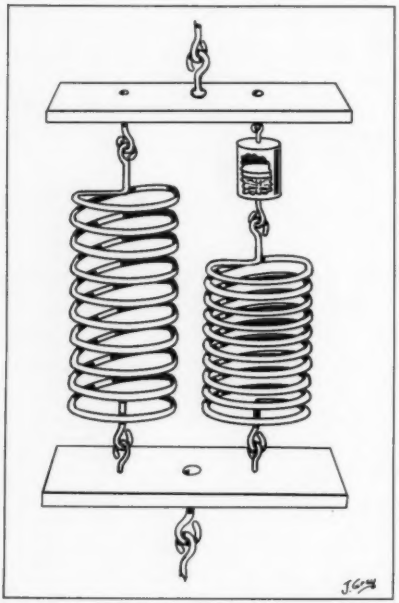


Figure 1

The so-called Zener model of anomalous elastic behavior. The figure represents a structural material loaded in tension. The material behaves as though there were several elements present. On the left is a major, perfectly elastic component in which strain is proportional to stress and in which no energy loss (technically known as *damping)* occurs.

On the right is a smaller, similarly perfect elastic component *in series* with a perfectly inelastic component (the dashpot). The dashpot resists elongation by virtue of the reduced orifice through which its hydraulic fluid must flow to permit elongation. It also resists return to original length when the load is removed. Finally, it dissipates energy as heat.

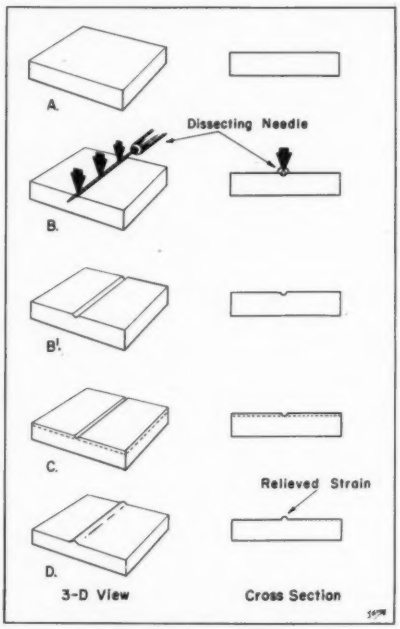


Figure 2

Schematic illustration of the events described in the text. The letters correspond to the steps following the same letter headings in the text under METHODS.

METHODS

A) Test samples were cut from the diaphyseal cortex with a fine toothed hack saw and the test sample surfaces ground flat and parallel under running water by methods described elsewhere.[7] The finished samples were about 10 x 5 x 5 mm. in dimensions.

B) The two rams described in figures 1 and 2 in the preceding paper were forced into the surfaces of the test samples by clamping them in a vise as previously described. The loads selected were sufficient to produce plastic flow as previously described. As a result a permanent set was produced in the surfaces of the test samples.

C) The indented surfaces of the test samples were then ground sufficiently under running water so that all evidence of the dents was removed, the finished surfaces being perfectly flat to inspection with optical aid in oblique light.

D) The samples were then subjected to one of the following manipulations:

1) Immersion in 95% ethanol for 10 minutes.

2) Immersion in pure dioxane for 10 minutes.

3) Immersion in 2% nitric acid for 4 minutes.

4) Boiling in water 10 minutes.

The test surfaces were then inspected again for surface flatness.

OBSERVATIONS

At the conclusion of step (B) a permanent set was visible in the surfaces of the test samples of bone. At the conclusion of step (C) the sample surfaces were perfectly flat. At the conclusion of steps (D) 1, 2, and 3 a hump had appeared on the surface of the test samples. The hump was a negative image of the original indentation, and a positive image of the shape of the ram producing the original indentation. The hump was considerably smaller than the depth of the original permanent set. See figure 2.

At the conclusion of step (D) 4 no hump was apparent on the surfaces of the test sample.

It appeared to be easier to elicit the above behavior on material loaded parallel to the longitudinal axis of the original diaphysis than on material loaded parallel to other axes.

DISCUSSION

The behavior observed above may be explained by inferring the following:

There are two elastic components present in fresh, wet bone. Under normal loads their effects are additive. Under abnormal loads one phase may be loaded into the region of plastic flow, and may be given a permanent set, at slightly lower loads than are needed to produce the same behavior in the other phase. The result is that the permanent set in the first phase "locks in" elastic strain from the second phase.

If the bonds holding the permanent set in the substance of the first phase can be broken, cancelled, or removed, the elastic strain of the second phase then corrects itself, producing its original shape and dimensions, or a hump.

It seems clear that phase one referred to above is the mineral in bone. This is the weaker phase which takes a permanent set before the matrix. Decalcification removes the mineral, while dehydration in some manner cancels the intercrystalline bonds. These bonds hold the permanent set of the mineral against the tendency of the elastically strained matrix to return to its original shape and dimensions.

It also seems clear that the matrix is the most durable phase which behaves elastically, and is phase 2.

It remains to be seen whether or not matrix can also be given a permanent set; the presently available data suggest that the permanent sets noted in the course of this work might all be due to the mineral phase alone. Not all of the samples tested developed humps after ethanolization. It is possible that in such samples matrix was given a permanent set too.

The manner in which ethanol and dioxane release the bonds holding the permanent set in the mineral phase may be significant. One theory is that the plastic flow in bone is a cog-wheel phenomenon, the cogs locking each other after plastic flow. The alcohol, by producing shrinkage, merely disengages the cogs. The other theory is that the alcohol, by virtue of its polar nature, attached to and thus blocked certain types of bonds holding adjoining crystal masses together. By blocking, or cancelling, the bonds, the elastic strain in the matrix would be released. If something of this order occurs, then one might expect to find that the strength of bone may be affected considerably by the type and number of nonhydroxyapatite ions in the bone. This is because such ions could reinforce or interfere with the bonding efficiency due to quantum effects.

If the shrinkage explanation is correct, bone's strength and rigidity should be found to vary according to the amount of water present in it. (This is an observed fact.) It is significant that bone is known to be dependent on both of the factors mentioned. One may infer, for purposes of designing future experiments, that there is some truth in both theories postulated to explain the release of the elastic strain in the matrix.

SUMMARY

By simple experiments a biphasic elastic behavior in wet, fresh bone can be elicited. The facts suggest that the mineral phase may be given a permanent set before the matrix, and that upon releasing the permanent set of the mineral by two means, the matrix returns to its original shape elastically. Two possible reasons for this behavior are mentioned, and the gross consequences to be expected if either reason is correct are predicted.

PHYSICAL CHARACTERISTICS OF BONE — PART III

A SEMIMICRO MEASUREMENT OF UNIT SHEAR STRESS

HAROLD M. FROST, M.D., HERBERT ROTH, B.A. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

There are three basic types of stress: tensile, compressive, and shear. Stress is the intermolecular resistance developed within the substance of a material which resists deformation under the influence of a load. Tension is a pulling apart, compression a pushing together, and shear the force experienced by a sheet of paper between the blades of a pair of scissors.[5,6,11]

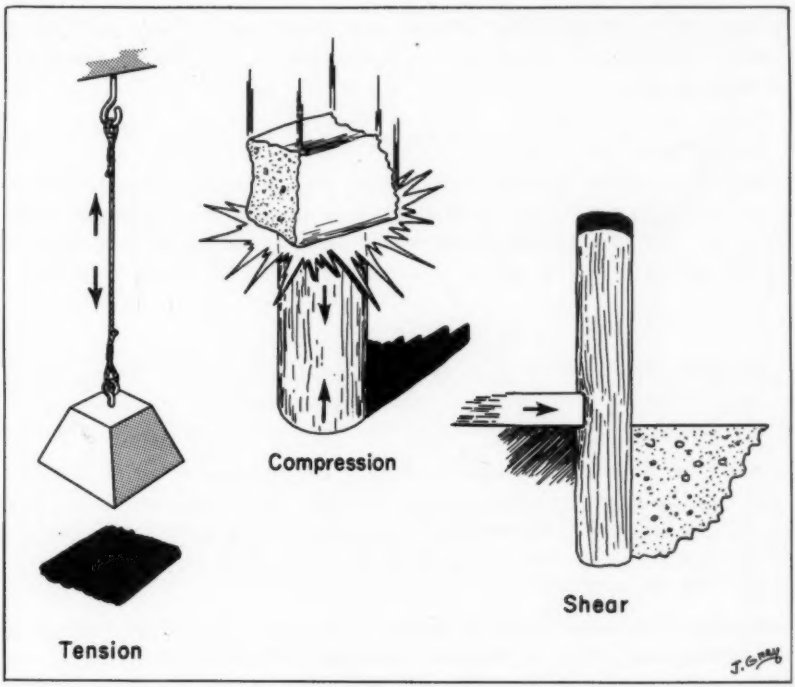


Figure 1

Schematic illustration of the three principle stresses. It is believed that *all* possible types of load may be explained in terms of these three types of stress.
Unit stress is the amount of stress on a unit area (such as 1 mm^2, 1 cm^2, 1 $inch^2$) cross section. The cross section is *normal* to the direction of tension and compression but is *parallel* to the direction of shear.

The shearing strength of human bone, both wet and dry, has been measured in a number of ways. Values averaging 8 Kg/mm^2 parallel to the longitudinal axis and 11 Kg/mm^2 transverse to the longitudinal axis have been obtained. The spread in the measurements is over 100%, necessitating that large numbers of measurements be averaged to obtain a significant result. The large spread in individual measurements

means the presence of uncontrolled variables. This has been recognized. Attempts to correlate variations in strength with variations in the volume of the vascular channels, with the orientation and density of the collagen bundles and with cement lines have been made or suggested, and produce modest improvements in results.[3,4]

To find the remaining variables, and to quantitate the variables, tests on microscopic volumes of bone must be done. Correlation of variability must be sought for with, in addition to the variables in the preceding paragraph, the amounts of micropetrosis and feathering, osteocyte death, cement line area per unit volume, water content per unit volume, size and numbers of Haversian systems per unit volume and, perhaps, the mineral composition of the bone.

Here a semimcro method for measuring unit shear strength is presented. Although the method has faults, it has pointed out how future work of similar nature should be designed.

MATERIALS

Two tibiae and two femurs were obtained fresh from the operating room, the amputations being done for soft tissue neoplasm in one case and vacular insufficiency in the other. The specimens were kept moist before and during the experiments with tap water applied by suitable means. The patients' ages were 18 and 63 years.

METHODS

Unstained, fresh, undecalcified, unfixed, unembedded sections 70 microns thick were made by methods described elsewhere.[7] Section orientation was such that shear load was parallel to the longitudinal axis or perpendicular to it.

Unit shear stress is the amount of shear stress developed in a unit area of the material. In this work cgs units are used so values are in mm. and Kg. The unit shear stress measured is that presumed to be present at the moment of failure. This presumption assumes too much; in precise work a number of variables not dealt with here must be controlled.

The shear test apparatus is illustrated in Figure 2. The arrangement is such that a circular ram 0.9 mm in diameter is punched through a hole of slightly larger diameter in an anvil lying under the ram. If a bone section is interposed between the ram and anvil, then a circular button or cylinder of bone will be punched out. The resistance to the punching action will, in theory at least, be shear and will occur in the wall of the cylinder rather than in its ends. The test apparatus is arranged so that load in the form of sand may be added at a rate of 1 Kg/minute. The total load on the ram at the moment of failure is measured by weighing the sand and container on a suitable balance. The mass of the device supporting the ram was separately weighed (97 grams) and was added to the totals to obtain the true weight on the ram.

The hinges in the device supporting the ram depend on the V-block principle for location.

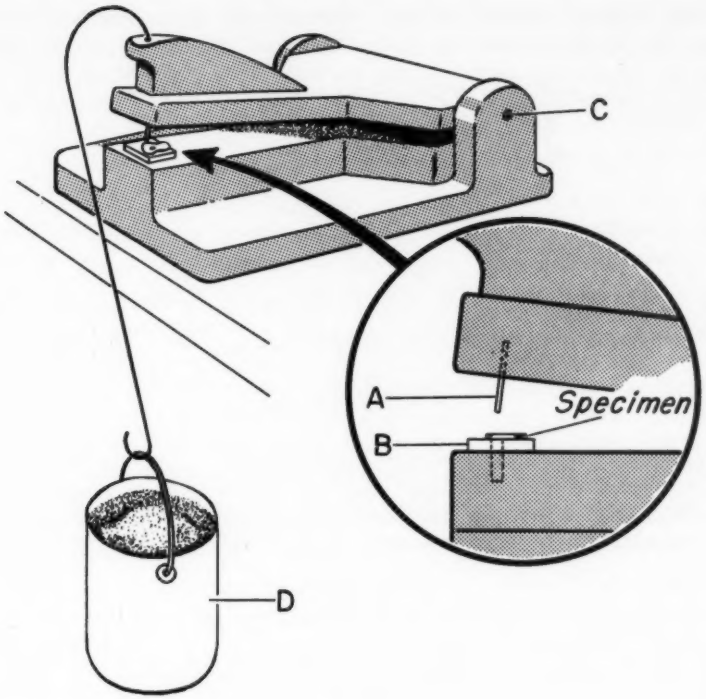


Figure 2

The shear test apparatus:
A is the ram, a dissecting needle 0.9 mm diameter.
B is the anvil, a metal plate with a hole drilled to accommodate the ram.
C are the hinges which utilize the V-block principle to maintain localization in space along the x, y, z axes.
D is a bucket into which sand is poured during tests.

In practice the anvil is centered and clamped under the ram so that the traverse of the latter leads it through the hole in the anvil. A section is then placed between the two and the ram allowed to rest on the surface of the section. The weight is then added to the weight carrier until the ram punches through the section. The weight is recorded, as is the location of the hole in the section under test. India ink marks or V-shaped notches are used to identify a reference mark on the section. Multiple holes are punched into each section at equidistant parts of the circumference or length. Sketches of each section were made and the holes and the loads noted appropriately so that at any time in the future values for any hole could be found.

Upon completion of the tests the sections were dried in air and mounted in H.S.R. on ordinary microslides. The diameter of each hole in the section was measured with a calibrated eyepiece micrometer. The thickness of the section around each hole was measured with the fine motion screw of an AO medical microscope. It was

necessary to measure thickness at three or more points a diameter or so removed from the edge of the punched out hole. The reason is that plastic flow had been produced by the ram, markedly thinning the bone at the edges of the hole in comparison to the average thickness of the surrounding section. The measured thickness is multiplied by 1.5, the refractive index of the bone, to correct for the distortion in optical depth caused by the refractive index.

The surface area of the wall of the cylinder of punched out bone is π D T, D being the diameter of the hole and T the thickness of the hole. This area is A, and is measured in mm^2.

The total load at the moment of shear failure is P, in kilos.

The unit shear stress at point of failure is $S_s = \frac{P}{A}$, and is Kg/mm^2.

OBSERVATIONS

In a few instances poor centration of the ram over the hole in the anvil led to excessively large shear values. These instances were discarded.

In about half of the tests microscopic inspection of the hole punched in the section revealed that one endge was quite ragged. Poor centration is suspected in

Table I

Specimens	Type of Section	Direction of Shear	Number of Valid Determinations	Average Unit Shear Stress Kg/mm^2	Maximum and Minimum
Femur	Cross	Longitudinal	3	7.56	8.8 Postquadrant 6.5 Ant. Quadrant
Tibia	Cross	Longitudinal	5	8.15	9.5 Postquadrant 6.4 Ant. Quadrant
Tibia*	Cross	Longitudinal	5	6.94	7.4 Lat. Surface 6.4 Crest
Femur	Longitudinal	Transverse	3	8.8	9.9 8.1
Femur	Longitudinal	Transverse	3	8.0	8.6 7.0
Average:	Cross	—	13	7.5	
Average:	Longitudinal	—	6	8.4	

*See Legend

LEGEND

The bone sampled is listed in the left column, and next to it the type of section tested. Punching a hole in a cross section is done by producing longitudinal shear. The direction of shear is thus the opposite of the type of section tested, and is listed in the third column.

A total of 27 separate measurements were made. Eight were discarded for reasons dicsussed in the text. The number from which the averages were made is listed in the fourth column.

The largest and smallest values accepted are listed for each specimen in the last column.

*This section was not cut at a true 90 degrees to the long axis of the bone, but at a 60 degree angle to the long axis.

these instances but there is no consistent variation of this group compared to the group with perfectly cut holes.

The spread of individual measurements, compared to the means, was plus or minus 15%, a considerable improvement over the spread affecting macroscopic measurements.

The results of the tests are listed in Table I.

The mean unit shear stress with this method is about 7.5 Kg/mm² perpendicular to the long axis.

Consistently less shear strength was observed in the crest of the tibia as compared to the posterior surface, and in the highly remodelled angles of the femur compared to the less remodelled surfaces. The majority of the apparent spread in individual measurements seen in Table I is the result of this effect, so that the spread in the data is actually much less than the 15% figure quoted.

The crest of the tibia contains more vascular channels, more Haversian systems, less circumferential lamellar bone, and more square millimeters of cement line per unit volume than does the posterior surface, as do the femoral angles compared to surfaces.

Due to the large size of ram used in these experiments — 0.9 mm — it was not possible to test individual Haversian systems, cement lines or areas of circumferential lamellae.

DISCUSSION

A) The small spread in the individual measurements means that miniaturization of the strength testing procedure is a productive approach. It should be carried further, as will be pointed out below.

B) The diameter of the ram — 0.9 mm — is too large. The ram should be small enough so that individual Haversian systems can be tested, or individual moieties of circumferential lamellar or micropetrotic bone. Based on measurements of human Haversian systems, a ram diameter of 15 — 200 microns would be better, useful, and feasible.

C) A smaller ram would require better centration of ram and anvil. This means a test device constructed of metal.

D) A round cross section of the ram in testing shear strength parallel to the long axis of the bone is quite useful, and in testing individual cement lines will be essential.

The round cross section is wrong for tests done perpendicular to the long axis because the circumference of the ram in one quadrant is applying shear parallel to the grain, while at 90 degrees distance it applies shear perpendicular to the grain. The result is an average, with no means of determing how far apart are the two extremes that are averaged.

For tests on longitudinal sections a ram that is flat, rectangular and markedly elongated will be required.

As a result of these factors the shear measurements on the longitudinal sections should be discounted.

E) It has been assumed in this and the previously described shear experiments that the idealized version of a true plane of shear occurs in the substance. In actuality this is not, and almost never is, so. The microscopic irregularities of any structural material produce microscopic rerouting of the stresses and strains within the material so that the physical behavior and the unit stresses at the microscopic level may be fantastically complex. This may also be the case with the simple shear experiments reported in this paper.

F) The method of preparing the sections for testing may produce some degree of surface damage which leads to a 5 micron thick surface layer that is much weaker than the remainder of the section. Damage of some sort is known to occur, because the ground surfaces are unusually permeable to fuchsin and other reagents, to a depth of about 5 microns. It will be necessary to look for this effect. It it exists, it must be corrected to obtain precise measurments.

G) The rate of application of load, and a reasonably instantaneous method of arresting load application, wlil need to be tended to in future experiments with smaller rams that are properly shaped. This attention is needed to obtain the precision promised by the miniaturization process.

SUMMARY

A semimicro measurement of unit shear stress at failure has been made with simple apparatus. The value of unit shear strength in shear parallel to the long axis of the bone is 7.5 Kg/mm^2.

From this work it has been learned how to improve the design of miniaturized physical testing in shear.

PHYSICAL CHARACTERISTICS OF BONE PART IV

MICROSCOPIC PREFAILURE AND FAILURE PATTERNS

HAROLD M. FROST, M.D., HERBERT ROTH, B.A. AND ANTHONY R. VILLANUEVA, B.S.

INTRODUCTION

When a load is applied to a structural member the member resists deformation by the load. Intermolecular forces are developed, are responsible for the observed resistance, and are known to the engineer as stresses. There are 3 principal stresses: tension, shear, and compression. These forces are diagrammed in figures 1, 2. All of the stresses developed in a material can be accounted for as combinations of the 3 basic stresses.[5,6,11]

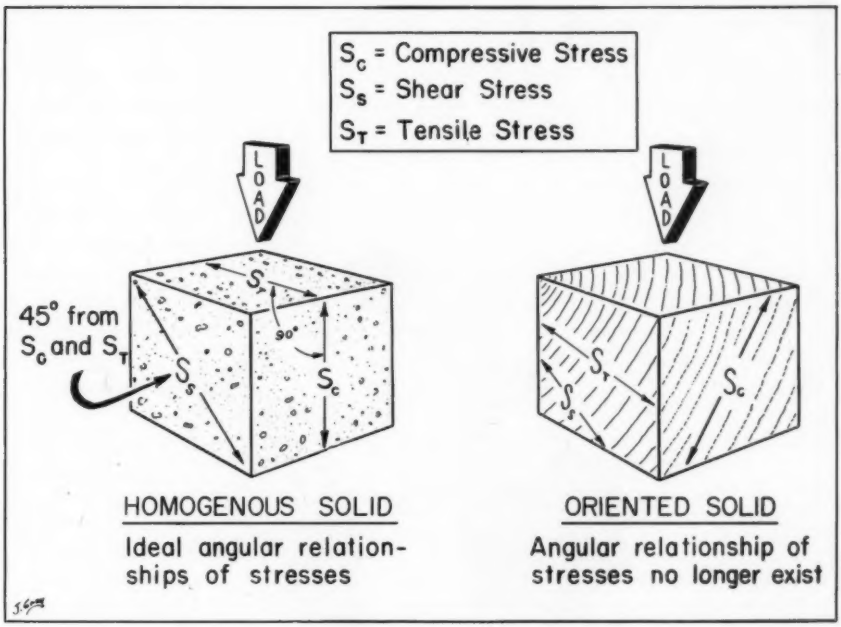


Figure 1

The figure on the left illustrates the three principal stresses. (These stresses are also diagrammed in figure 1 in the paper on unit shear stress in the preceding paper). The compressive load applied to the top of the homogeneous solid produces components of compressive, tensile and shear stresses in the interior of the solid which resist deformation by the applied load. Shear is normally oriented 45° to the maximum tensile and compressive stresses. Tension and compression are normally oriented at 90° to each other. In a two-dimensional analysis there is only one direction for maximum tension and compression but there are two directions for maximum shear.

On the right the effect of the presence of grain in the structural material is daigrammed for a block of wood. Wood is qualitatively similar to bone in many respects. The direction of the maximum tensile, compressive and shear stresses no longer bears the ideal angular relationship present in the left of this figure. The actual relationship must be determined empirically for each new substance. The existence of this effect has not been previously pointed out as far as bone is concerned.

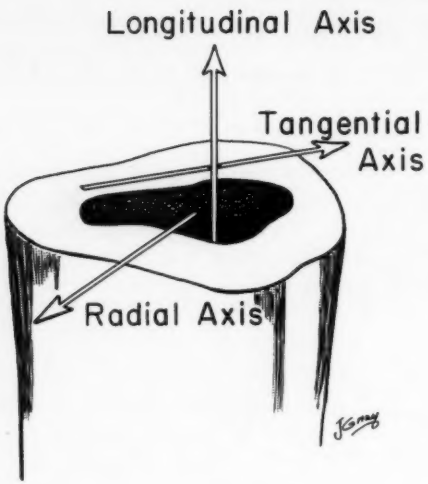


Figure 2A

Diagrammatic presentation of the meaning of the various axes referred to in this and the preceding papers.

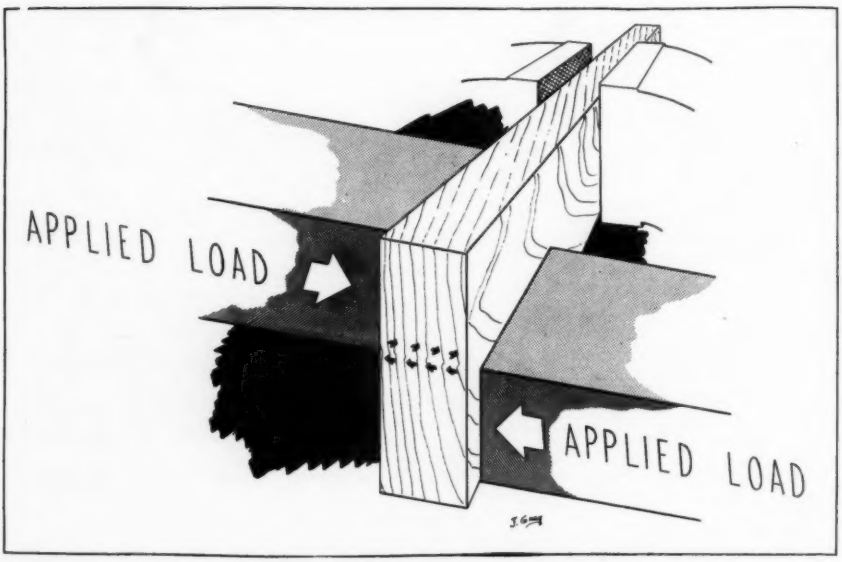


Figure 2B

Diagrammatic representation of shear. Of the three principal stresses, this is the hardest to grasp. The two opposing loads illustrated would tend to split the board across horizontally, along the plane designated by the small arrows in the center of the end face of the plank. The forces developed within the interior of the board which resist the applied load is shearing stress. When the unit shear stress exceeds the strength of the material, fracture will occur.

In an ideal, homogeneous structural material the various stresses bear a consistent relationship to each other, assuming that the structural member is under only one exterior load. The principal tensile and compressive stresses act at 90° to each other

and in only one direction each. Shear however acts at 45° with respect to both tension and compression, and there are two directions of shear possible.[5,6,11]

In most real structural materials there are varying degrees of inhomogeneity. Inhomogeneity is not always undesirable; forging of metals is done to orient inhomogeneities in such a way that strength is improved in desired directions, while the grain of wood and bone impart desirable strength characteristics along certain directions which are useful to the organism.

Structural inhomogeneity affects the distribution of the stresses and strains resulting from an applied load. They may no longer bear consistent relationships to each other. It is even possible that there be set up two or more sets of principal stresses at the microscopic level, especially if the material is composed of two major phases with considerable orientation imposed upon them. This is the situation found in bone.

It would seem desirable to observe in some manner the reaction of bone at the microscopic level to varying loads.

Fortunately a method for making such observations exists. The method depends upon the empirically observed fact that when loads upon a moiety of bone are just short of the point at which gross failure or cracking occurs, something happens to the impermeability of the mineral in the bone. Normally mineralized bone is so impermeable that the usual stains utilized in work with undecalcified sections do not permeate the bone in the sense that a blotter is permeated. The walls of the various holes normally found in bone are stained but the bone substance itself remains as clear as glass.

Under loads just short of failure, normal stain impermeability disappears. The bone stains diffusely. If the concentration of damage is along a plane, then the stains will appear along a plane. If the concentration is diffuse, then the stain will be diffuse.

The fuchsin permeability produced under loads just short of those required to produce gross cracks are termed *prefailure planes* in this laboratory. These prefailure planes may be used to study the patterns of prefailure loads under varying conditions and in varying types of bone. While it is felt at present that the prefailure planes are shear failure planes, the reader must keep in mind that this is not yet wholly proven. They might be tension or compression failures too.

MATERIALS

Two tibiae and two femurs were obtained fresh from the operating room, the amputations being done for soft tissue neoplasm in one case and for vascular insufficiency in the other. The specimens were kept moist before and during the experiments with tap water applied by suitable means. The patients' ages were 18 and 63 years.

METHODS

Test samples were cut from the diaphyseal cortices with a fine toothed hack saw and the surfaces ground flat and parallel under running water by methods described elsewhere.[7] Sample size was about 10 x 5 x 5 mm.

A shear load was then applied to the test samples by clamping them in a vise between the ram and anvil described in Figure 1, Page 150 this series. For the present purpose loads in the range required to produce plastic flow and a permanent set were selected. It was not necessary to produce plastic flow to produce the phenomena about to be described.

The samples were left clamped in the vise jaws between ram and anvil for periods ranging from a minute to 24 hours. Then the samples were removed. Thin, undecalcified, undehydrated sections were then cut through the samples in such a manner that the edge of the finished sections was cut through the face resting on the ram. These sections were then stained with basic fuchsin, reground and mounted for observation and photography by methods described elsewhere.[8]

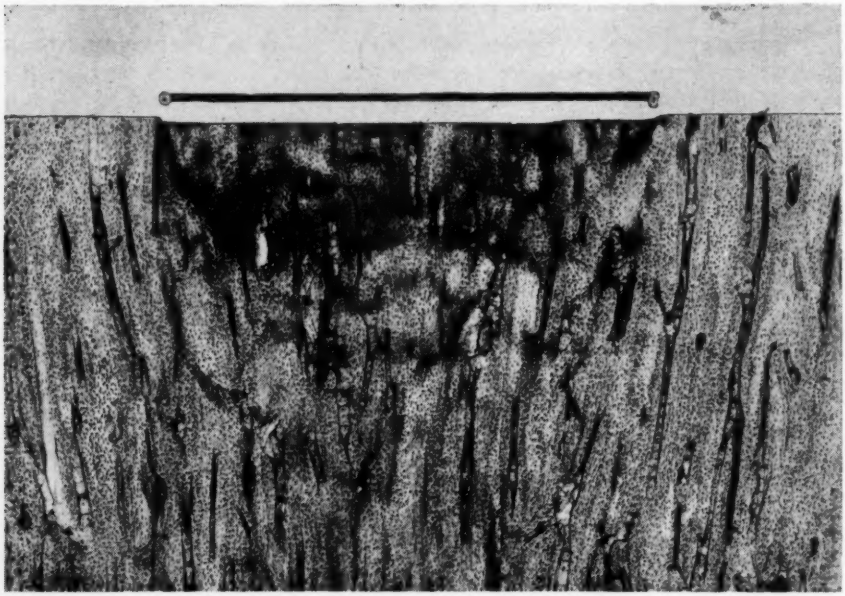


Figure 3

Longitudinal section through loaded test specimen stained with basic fuchsin. About 50 x. The India ink line above the edge of the section (top) depicts the width of the ram which applied the load. The depression of the section edge under this line is the permanent set produced by the load. The darkness of the section immediately under the permanent set is due to the presence of basic fuchsin in prefailure planes.

Note that deeper in the section from the surface loaded by the ram, there are no visible prefailure planes.

Specimens were loaded and cut so that the final sections were either longitudinal or cross sections of the diaphysis. In the longitudinal sections shear had been produced parallel to the long axis of the bone while in the cross sections shear had been produced parallel to the radial of the bone.

OBSERVATIONS

SHEAR PARALLEL TO THE LONG AXIS

Figure 3 illustrates the edge of one of these sections. The indentation of the ram may be seen along the upper edge of the section. The seemingly smudged areas deep to the ram's indentation are prefailure zones stained with fuchsin. Because these prefailures zones lie parallel to the plane of the paper they lack definition.

Figure 4 illustrates a higher power view of the left edge of the section in Figure 3. At 4 o'clock there are a number of lines which intersect with each other

Figure 4

150 x. View of left hand edge of the permanent set illustrated in Fig. 3. The step-off at the top of the section is at the edge of contact with the ram.

In the lower right the criss-cross planes of prefailure described in the text may be seen.

The small, black spots are osteocyte lacunae. The elongated dark structures are Haversian canals in longitudinal section.

Figure 5

View of different section of different test specimen than figure 4. 250x. The step-off at the right edge of the section is where the edge of the ram lay.

Fanning out from this edge downward and to the left are a number of pre-failure planes which, as depth increases, are increasingly crossed by other prefailure planes taking the form of mirror images.

While there is only *one* direction of principal tensile or compressive stress in a material, there are always *two* directions of principal shear stress. This *suggests* therefore that the criss-cross mirror image pattern seen here represents shear prefailure instead of tension or compression prefailure.

at an angle of about 30 degrees to the longitudinal axis of the bone and section. These are also prefailure zones but in this case they lie perpendicular to the plane of the paper and so are most sharply defined. Their width is on the order of 10 microns. Even at highest optical resolving power there is no visible structural feature in the prefailure planes. The discontinuities which permit them are of ultramicroscopic dimensions.

Figure 5 illustrates the right edge of a similar test sample with similar appearance of prefailure planes. The planes begin at the edge of the ram which loaded the specimen. The intersection angles are the same as in figure 4.

Figure 6

150 x. View of right side of region of permanent set section illustrated in figure 3. In the upper part of the Haversian system lying on the right side of the figure some prefailure planes parallel to the long axis of the bone, and also to the direction of loading, may be seen. Two India ink marks identify them. This was what we expected to see, but saw disconcertingly little of!

Prefailure planes more or less parallel to the plane of the section darken the area of bone under the edge of the ram at the top of the figure.

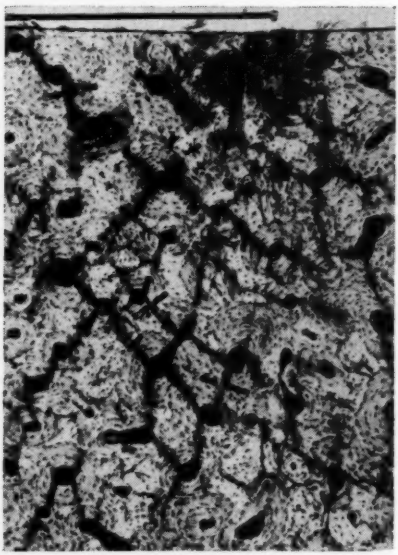


Figure 7

80 x. Cross section. At the top edge of the section an India ink line shows where the ram contacted the surface of the original test specimen. The idealized shear load applied by the ram lay in a plane defined by one line dropping vertically from the right edge of the ram to the bottom of the figure, and by another line perpendicular to the plane of the paper.

The small, black circular areas are Haversian canals seen in cross section. Connecting a great many of these canals are rather broad prefailure planes which are perpendicular to the plane of the paper. For clarity several are identified between India ink marks.

This test specimen contained considerable amounts of micropetrotic bone.

Figure 6 is a high power view of the right edge of the section in Figure 3. At 2 o'clock a fine, longitudinal fibrillation pattern of prefailure zones is seen. This zone lies just to the left of the Haversian canal seen in longitudinal cut on the

right of the figure. The longitudinal prefailure pattern shown was visible on many of the test samples.

SHEAR RADIAL TO THE LONG AXIS

Figure 7 illustrates a section cut through a test sample loaded in shear parallel to the radial axis. The section is a cross section of the diaphysis. The plane of shear produced is perpendicular to the plane of the paper and runs from the top to the bottom of the illustration.

The curious and striking thing about the prefailure zones stained with fuchsin in this and similar specimens is their direct course from one Haversian canal to another. The average direction of the prefailure planes is 45 degrees to the vertical and to the plane of the applied shear load. In no case does the prefailure plane run parallel to the idealized plane of shear, although one prefailure plane closely approaches it.

The thickness of these prefailure planes is about 50 microns, considerably more than the thickness of the prefailure planes seen when shear was applied parallel to the long axis of the bone.

When too much clamping force was applied some cracks were produced in the test samples and may be seen in the finished sections. These cracks usually coincide with the prefailure planes, often occurring through prefailure planes.

DISCUSSION

Recall that while the principle tensile and compressive stresses in a material exist in only one direction in an ideal homogeneous solid, the principle shear stress exists in two directions.* Referring to Figure 5, these two directions are down and to the left, and down and to the right.

For the above reason we feel that the prefailure zones stained with basic fuchsin are probably shear failure zones, rather than incipient failures in tension or in comparison.

A) Using fuchsin permeable prefailure zones as an index, it may be inferred that the distribution of shear stresses in a fresh, wet, human bone sample loaded in shear is anything but simple or ideal. It appears unexpectedly complicated and as though it has been reoriented within the section by some feature of construction of the bone.

B) Additional work is required, designed around the features revealed in this work, to be certain of what is occurring in the shear loaded samples.

C) We wish to note that Rutishauser has observed the criss-cross pattern revealed in Figure 5. He found it as a system of haematoxylin-staining cracks in dog bone and interpreted these cracks as existing in vivo. Unfortunately the methods of preparing sections which he used compel us to doubt that the observed cracks or incipient cracks existed in vivo. A considerable experience with in vivo cracks in human bone

makes us doubt that such a criss-cross pattern is the one usually found in vivo micro-cracks. The micro-cracks we find are usually smaller, fibrillated, but single.

Nevertheless the pattern of cracks noted over 8 years ago by Rutishauser now appears again. The pattern indicates some fundamental physical attribute of bone under shearing load. When the exact nature of this physical attribute is known, a great number of additional facts will also have been added to our knowledge of bone. It is remarkable that the implication of Rutishauser's observations has remained unattended for so long, and is concrete evidence of how badly the orthopedic basic sciences need more research.[12]

REFERENCES

1. Bechtol, C. O., Ferguson, A. B., Jr., and Laing, P. G.: Metals and Engineering in Bone and Joint Surgery, Baltimore, Williams & Wilkins Co., 1959.

2. Cottrell, A. H.: Theorectical Structural Metallurgy, ed. 2, London, Edward Arnold Ltd., 1955.

3. Evans, F. G.: Stress and Strain in Bones, Springfield, Thomas, 1957.

4. Evans, F. G.: Relations between the microscopic structure and tensile strength of human bone, Acta Anat., Basle 35:285, 1958.

5. Eshbach, O. W., ed.: Handbook of Engineering Fundamentals, ed. 2, New York, John Wiley, 1958.

6. Frankel, J. P.: Principles of the Properties of Materials, New York, McGraw-Hill, 1957.

7. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

8. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

9. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

10. Goldman, J. E., ed.: The Science of Engineering Materials, New York, John Wiley, 1957.

11. Olsen, G. A.: Strength of Materials, ed. 2, Englewood Cliffs, New Jersey, 1956.

12. Rutishauser, E., and Majno, G.: Physiopathology of bone tissue: The osteocytes and fundamental substance, Bull. Hosp. Joint Diseases 12:468, 1951.

*This is true in a two dimensional analysis; when a three dimensional analysis is made the real stress orientations are more complex.

OBSERVATIONS ON PASSIVE TRANSFER OF BONE MINERAL IN MAN AND NUCLEATION

HAROLD M. FROST, M.D.

INTRODUCTION

Much work and writing in the last five years indicates that mineralization of human bone matrix is probably a biphasic process. The theory is as follows:

1) The first phase is known as nucleation. As summarized by Glimcher[9] and Neuman,[11] nucleation is the formation of the nucleus or base of a crystal in the bone matrix. The nucleus involves a few of the ions characteristic of the hydroxyapatite lattice, depends on specific chemical and steric attributes in the matrix, and follows some specific but unknown chemical "maturation" process in newly formed matrix. The formation of the nucleus may involve an organic epitaxy.

2) The second phase of bone mineralization has not been named. Here it will merely be called mineralization to distinguish this phase from the nucleation phase described first.

Bone mineralization in normal body fluids proceeds independently of the action of a living cell because, in terms of activity coefficients, the body fluids are supersaturated with respect to the mineral crystallites. The presence of preexisting hydroxyapatite crystals seems to be all that is needed for the deposition of additional crystals.

3) The ability to calcify is characteristic only of certain tissues in the body. These tissues are teeth, hyaline cartilage, and bone. The specific feature or features which permit these tissues to mineralize are to be found in the cement substances of each tissue; the collagen appears to be structurally and chemically the same in all three and similar to the collagen in the remainder of the body's non-mineralizing tissue.[9]

In this paper certain simple observations are presented which bear to some extent on the theoretical model just outlined. The observations are fortuitous rather than experimental.

OBSERVATIONS AND DISCUSSION

PASSIVE TRANSFER OF MINERAL

In this laboratory fresh, undecalcified bone sections are the major investigative material. The sections are made and stained by methods which produce less artifact in sections than is produced by any other existing sectioning method.[3,4] Human biopsy specimens prepared by these methods have been kept alive for several weeks in tissue culture.

When fresh, wet undecalcified, unfixed, unembedded human bone is stained with 1% basic fuchsin in 40% ethanol, the resulting stain reveals two things. First, all holes normally found in bone are stained red.[1,4] Second, and more important for the present purpose, all bone mineralized below about 80% of its maximum is fuchsin permeable, and thus a diffuse red similar to an automobile tail light roundel. All

bone mineralized above 80% (this is only roughly measured) is impermeable to the stain and appears as clear and colorless as clear glass on finished sections. The holes in the bone are clearly visible.[1,4]

With the aid of these methods a new bone disease termed feathering[1] has been discovered. (Fig. 1) Feathered bone is incompletely mineralized morphologically, and markedly delayed in its rate of further mineralization dynamically. Some skeletons contain impressive proportions of feathered bone.

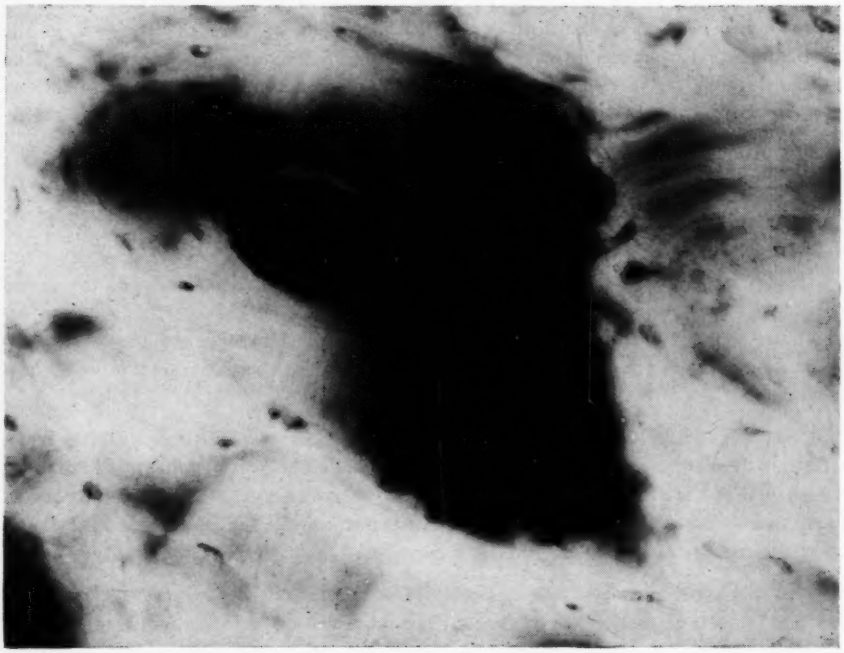


Figure 1

Fresh, undecalcified human clavicle about 200 x, cross section, basic fuchsin, Wr. 58. The Haversian system in the center is feathered. The black portion is heavily stained with fuchsin due to low degree of mineralization. A clear, highly mineralized ring lines the Haversian canal. The disease stops at the cement line.

There are several cases in the case material obtained by the laboratory with very severe feathering revealed by the initial sections. Over 9 months after these specimens were obtained, and after the initial fresh sections had been prepared, it was decided to make an additional batch of sections for resident teaching purposes. The new sections were prepared from bulk material such as rib or clavicle that had been stored at room temperature in 40% ethanol.

The new sections contained markedly less fuchsin permeable bone than the original sections in every one of the four cases in which this phenomenon was sought for.

We must infer that during the months of shelf life, mineral in areas of high mineral density redistirbuted to areas of low mineral density within the bone. There was no outside source of mineral; the specimens were kept in glass jars with air tight seals.

This simple observation means several things.

A) In vitro, areas with low mineralization per unit volume of bone possess lower free energy than areas with high mineralization per unit volume of bone. The transport of mineral observed in vitro must have occurred at the expenditure of energy falling down a gradient which originally existed in the bone. This will be recognized as a thermodynamic concept.

B) If considered as an isolated non-living system, wet bone that is undamaged by embedding, fixation, heating, or the like tends to equilibrate the continuous spectrum of mineral densities normally present in a bone.

Figure 2

An osteoid seam as revealed in fresh, undecalcified sections. The seam lies between the bar markers. About 500 x. The upper edge of the seam is the wall of the vascular channel. The lower edge of the seam is advancing wall of mineralization referred to in the text. The lower half of the figure is mineralized bone. The direction of growth and of mineralization is upwards.
Reprinted by permission from Henry Ford Hospital Medical Bulletin.

C) The presence of an unstable energy gradient at the time the bone samples were placed in the jar for storage implies that the gradient was originally created

by living cells. This in turn implies that during life the cells maintain such gradients. At present there would seem to be as much reason to expect the cell to create zones of low free energy as there is reason to expect the cell to create zones of high free energy. In other words, the bone cells could either be pumping mineral into bone against a gradient, or pumping mineral out of bone against a gradient. These are the two possible situations which reason and data must eventually permit us to choose between.

D) In vitro in intact bone in an isolated system, progressive mineralization of already partially mineralized bone occurs.

PROBABLE SITE OF NUCLEATION AND MATURATION IN HUMAN BONE IN VITRO

Previously reported work recorded the fact that human lamellar bone normally forms first as an osteoid seam laid down on preexisting bone. Osteoid seams are not usually an abnormal feature of human bone, as is often taught, but are a normal

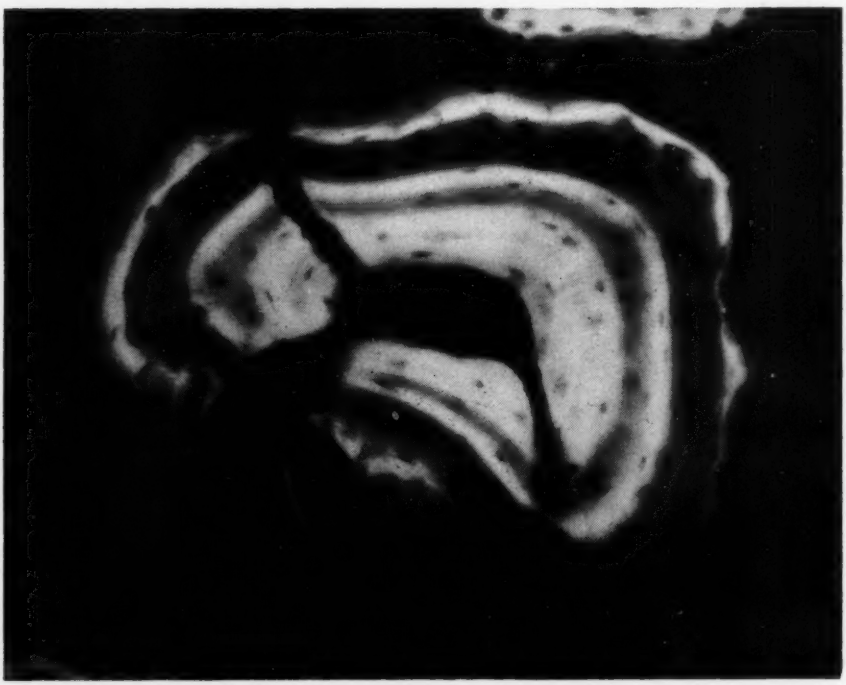


Figure 3

Cross section femur 48 year old man, fresh, undecalcified. Urinary tract infection 1956, treated with tetracycline intermittently. Amputation 1960 for tibial neoplasm. The bright bands are tetracycline, fluorescing with the writer's microfluorescence set-up, in an Haversian system. Each band represents a different time and duration of drug dosage. When the times and durations are known, the remodelling rate 4 years before amputation can be measured. New osteoid is deposited at about 1 micron per day. It is mineralized at the same rate. The tetracycline is deposited and in some manner fixed in situ where the osteoid begins to mineralize.

accompaniment of normal growth and normal skeletal remodelling.[2,6,10,11,12] Their presence in abnormal numbers, and in abnormal widths, are abnormal, but this is a separate story. (Fig. 2).

The simple facts above mean this: the only places in the lamellar bone skeleton of the human child and adult where the nucleation process described earlier occurs is in osteoid seams. The remainder of the skeleton is by definition, and in fact, already mineralized to varying degrees and so has already passed through the stage of nucleation. *Therefore, to study nucleation in human bone, osteoid seams must be studied.*

While much has been written about osteoid seams from the standpoint of osteomalacia, little has been written about their normal evolution and change. Here however the tetracycline bone labelling phenomenon reveals a great deal about human osteoid seam physiology in vivo. (Fig. 3).

The tetracycline bone labelling phenomenon is discussed at length elsewhere.[7,8] In brief, any of the tetracycline antibiotics present in the blood are deposited on all available bone surfaces and in the peripheral zone of osteoid seams where mineralization is beginning. When the tetracycline is discontinued, the surface stain in the skeleton disappears within 48 hours. The tetraycline desposited at the periphery of the osteoid seam where mineralization of osteoid commences remains permanently in place, the maximum observed time being 9 years as of this writing. The tetracycline fixed in the mineralizing osteoid does not diffuse in vivo or in vitro in fresh, undecalcified sections. The skeletally fixed tetracyclines can be detected and measured with the aid of a simple microfluorescence set-up.[5]

Any new bone forming during the administration of a tetracycline antibiotic is labelled by the tetracycline. It remains in situ until resorption of the bone occurs. Accordingly it is possible to measure the amount of new bone formed in unit time in a unit volume, and to observe where this activity occurs and is most or least active.

The reader will recognize a powerful, new and economical technique for investigation of human bone physiology in vivo. This possibility, long a dream, is now reality.

Observation of an extensive amount of human tetracycline labelled material has brought to light the following facts pertinent to the present presentation:

A) A small arbitrary unit volume of osteoid is formed by an osteoblast.

B) The unit volume of osteoid exists as osteoid for about 10 days, during this time being buried by additional new osteoid deposited each day.[6,8,12]

C) At about the 10th day mineral suddenly appears in the unit volume of osteoid. In a 4 day period about 75% of the total amount of mineral it can ever hold is deposited. The remaining 25% of the total possible deposit of mineral is deposited at an exponentially slowing rate for the remainder of the biological life of the matrix.

D) Under the light microscope an osteoid seam in an Haversian system appears as a ring of osteoid lining the Haversian canal, and thus separating the canal from

the mineralized bone. The periphery of the osteoid seam is the zone of osteoid which is about 10 days old, which is rapidly mineralizing, and which is creeping centripetally towards the center of the Haversian canal at the same rate as new osteoid is added each day by the osteoblasts lining the inner wall of the seam.

On the basis of the above facts the following may be inferred:

The mysterious chemical maturation in the osteoid seam which permits mineralization, and which is supposed to lead to nucleation, must occur either in the zone of initial mineralization (which is about 4 micons thick) or slightly in advance of this zone. The nucleation process in human bone, if it occurs (and there is good although not compelling evidence to support its existence), must occur at the inner surface of the advancing wall of mineralization at the periphery of the seam.

Therefore, if maturation of the matrix and nucleation are to be studied in human bone, attention should be directed to the normal osteoid seams of children and adults. Attention should be limited to lamellar bone because fibrous bone formation is not normally found in healthy humans.

The fact that tetracyclines are permanently deposited only in newly formed, progressively mineralizing lamellar bone (actually also in mineralizing fibrous bone and hyaline cartilage), and not permanently deposited in any other part of the lamellar skeleton, even though it be permeable to tetracyclines in vivo, strongly suggest that the mechanism of permanent fixation is associated either with the maturation process, or the nucleation process, or both. This means that the tetracyline labelling phenomenon may be the key needed to unlock the secrets of the maturation and nucleation processes.

SUMMARY

1) Equilibration of mineral densities in dead human bulk bone in closed systems over several months has been observed. The cause of the phenomenon is conveniently described in thermodynamic terminology.

2) Knowledge of human osteoid seam physiology indicates that the chemical maturation of bone matrix and the nucleation process leading to mineralization of human lamellar bone probably occur at the periphery of osteoid seams. Study of the peripheral zone, which is 4 microns wide, would yield more information about the maturation and nucleation processes than study of the remainder of the skeleton. The key to the study of the maturation and nucleation processes may be tetraycline fixation in the maturing and mineralizing osteoid.

REFERENCES

1. Frost, H. M.: A new bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

2. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

3. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

4. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

5. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

6. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

7. Frost, H. M., Villanueva, A. R., and Roth, H.: Tetracycline staining of newly forming bone and mineralizing cartilage in vivo, Stain Technol. 35:135, 1960.

8. Frost, H. M., Villanueva, A. R., and Roth, H.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

9. Glimcher, M. J.: Specificity of the molecular structure of organic matrices in mineralization, In American Association for the Advancement of Science: Symposium on Calcification in Biological Systems, ed. by Reidar F. Sognnaes, Washington, D. C., The Association, 1960. p. 421.

10. LaCroix, P., Ponlot, R., Dhem, A.: Recalcification in vitro de l'os compact décalcifié, Experientia 14:396, 1958.

11. Neuman, W. F., Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

12. Ponlot, R.: Le Radiocalcium dans l'Etude des Os, Bruxelles, Ed. Arsica, 1960.

13. Vincent, J.: Recherches sur la constitution du tissu osseux compact, Arch biol., Par. 65:531, 1954.

EVIDENCE FOR THE EXISTENCE OF TWO PHYSICAL STATES OF BONE MINERAL

HAROLD M. FROST, M.D.

INTRODUCTION

Our concepts of the chemical and physical nature of the bone mineral have undergone drastic changes in the last 25 years.

Originally the bone mineral was thought to be tricalcium phosphate. Then appreciation of the presence of differing ratios of calcium to phosphorus, and the presence of adventitious ions such as carbonate, citrate, sodium, and chloride, made this concept appear inadequate. After much work and much disagreement, a new concept of the bone mineral has evolved.

The new concept is that the mineral is present as hydroxyapatitie crystals. The crystals have attached to them hydration shells of water, and by various means noted elsewhere, the amounts of calcium and phosphorus, and the variable amounts of other ions, are accounted for. The route by which hydroxyapatites are formed in the bone mineral are at present unknown and the subject of considerable investigation.

In this paper I will present evidence which suggests strongly that in addition to the hydroxyapatite present in bone, for which compelling evidence exists, there is another physical state of mineral salts present. The structure of these salts and their chemical composition are unknown.

OBSERVATIONS

Several publications of this laboratory deal with the presence of a quasipermeable state of the bone surrounding living osteocytes. This quasipermeable state has been termed "halo volume." Another peculiarity of halo volume has been noted in versene decalcified, haematoxylin stained sections.

The oustanding technical peculiarity of the quasipermeability of the bone in halo volume is its disappearance in vivo after death of the osteocyte, and its rapid disappearance in vitro after drying and other manipulations that are common in histological work.

The peculiar sensitivity of the halo volume permeability was not recognized at the same time as the halo volume feature itself. The variability in demonstration of halo volume resulting from this ignorance necessitated delay of publication for a full 5 years.

DISCUSSION

The reproducibility with which halo volume may be demonstrated in perfectly fresh bone, and ablated by such a simple thing as drying sections for a few minutes in air, indicates that something about the physical state of the mineral in the halo volume moiety of the skeleton is different from the mineral in the rest of the skeleton.

Then non-halo volume skeletal moiety does not exhibit the permeability or loss of permeability characterizing halo volume.

If halo volume mineral were all hydorxyapatite in vivo, the peculiar behavior of halo volume would not be expected and could not be explained. If however there were present a mineral state — in adidtion to hydroxyapatite — which spontaneously converted to hydroxyapatite upon death, drying and other manipulations known to ablate the halo volume permeability, the observed phenomena would be accounted for.

Hydrated calcium phosphate immediately comes to mind since it is known to follow this observed behavior in vitro.

This line of speculation may be solidified somewhat by some additional observations. Publications in this laboratory have already noted the normal existence of halo volume in perfectly fresh human bone, and the fact that halo volumes in vitro enlarge in acid pH and shrink in alkaline pH. As Nordin has postulated, the pH of the living, normal, human osteocyte's lacunar fluid may well be about 6.8, rather than the 7.4 characterizing the rest of the body fluids. The high degree of diffusion impedence existing between the average osteocyte and its average source of blood supply supports this contention because it explains how such a variation would be possible.

Is the non-hydroxyapatite mineral fraction in the halo volume really a calcium phosphate? It will take considerable additional work to answer this question.

SUMMARY

The existence of halo volume and its peculiar behavior in vitro and in vivo make it necessary to infer a difference in halo volume mineral composition as compared to non-halo volume mineral.

KNOWN QUALITATIVE VARIABLES IN HUMAN BONE

HAROLD M. FROST, M.D.

Not long ago it was possible to state that bone was either normal or abnormal on the basis of measurement of the ratio of its weight dried at constant temperature, (on the order of 110 C) to the weight of ash remaining after burning off the organic part of the bone sample (at temperatures on the order of 800 C). Qualitative abnormalities of the bone were considered present if there were variations in the weight/ash ratio beyond certain empirically determined limits.

Recent work has brought to light a group of abnormalities in quality of bone which escaped detection by the above and other methods. In this paper some of these qualitative abnormalities are presented, in addition to the originally known abnormalities. The purpose of the presentation is to acquaint investigators of qualitative features of bone with these variables. While it is unfortunate that the additional variables will complicate future qualitative measurement of bone, the additional knowledge gained thereby may be expected to shed more light on skeletal physiology in the future.

QUALITATIVE VARIABLES

Some of the variables which are presented may be readily quantitated, others not, at least at present.

1) *Variation in mineral density.* A given unit volume of bone matrix may contain very little mineral, as in osteoid seams, or a great deal of mineral, as in micropetrosis, these being extremes. A given moiety of bone undergoes a definite and orderly progression of mineral accretion during its biological lifetime. A number of diseases may affect this orderly progression, leading to qualitative abnormalities.

2) *Variation in water content.* A given unit volume of bone matrix may contain only so much water. When the matrix is first formed and is completely unmineralized the water content is maximal. When maximum mineralization has been achieved the water content is minimal. In other words, water and mineral vary inversely, the amount of and volume of matrix remaining constant as far as is known at present. The significance of this shift is that water content may be used to study mineral content of bone. Water content is more easily studied than is mineral content.

3) *Average biological age.* In any given skeleton there is an average age of the myriad of bone moieties which comprise the whole. Recent measurements indicate that in ribs and clavicles this age is about 15 years at age 30, and 8 years at age 60 in man. The significance of the average skeletal age is that the average diffusion impedence in the mineralized matrix depends on the average mineralization of the bone. Increasing mineralization occurs with increasing age of any given bone moiety, and increasing mineralization increases diffusion impedence between blood and bone. The average age of the skeleton is dependent on the rate of remodelling.

4) *Average diffusion impedence.* As noted above, this is directly related to the age of a moiety of bone, older bone being more highly mineralized and more

impermeable to ions and molecules of all sizes. Diffusion impedence and degrees of mineralization are in turn affected by other variables, among them being osteocyte death in vivo, feathering, micropetrosis. Exchange between bone and blood of a number of bone seeking radioisotopes would be governed in large part by the average diffusion impedence of the skeleton.

5) *Feathering.* A newly recognized bone affection characterized morphologically by an abnormally low *degree* of mineralization of the affected matrix, and dynamically by markedly retarded *rate* of mineralization of the affected matrix. Feathering is not usually related to any known osteomalacic process. Presumably feathered bone is unusually permeable in vivo. On occasion over 75% of the skeleton is composed of feathered bone.

6) *Micropetrosis.* Another newly recognized bone affection, this time characterized morphologically by death of the osteocytes in the affected bone and occlusion of the canaliculae by dense, impermeable deposits of mineral. This disease is found with increasing incidence in older patients and in the presence of vascular insufficiency. The affected bone is highly mineralized and in vitro exhibits high diffusion impedence.

7) *Osteocyte Death in Vivo.* With advancing age an increasing proportion of one's store of osteocytes die off, leaving behind them empty lacunae. There are sound reasons for anticipating that bone in the region of such empty lacunae exchanges to a lesser degree with the blood than bone which contains live osteocytes. In addition the diffusion impedence in bone, most of whose osteocytes are dead, may be expected to be high because one of the dynamic factors normally opposing a high diffusion impedence is the system of concentration gradients produced by living osteocytes.

8) *Osteoblastic Activity.* It has recently become possible to measure human osteoblastic activity by two methods, both possessing considerable precision when compared to the methods previously available. It is now possible, in man and with certain limitations, to measure the amount of new bone formed per unit volume of skeleton per unit time. This is a true measurement of rate.

9) *Osteoclastic Activity.* It has recently become possible to measure osteoclastic activity in man also, again with useful precision. The method, and the results thereof, are not published but will be the topic of the next large group of publications from this laboratory. When a measurement of both osteoblastic activity and osteoclastic activity has been made, the results may be expressed both as the remodelling rate and in terms of skeletal balance.

10) *Mineral Composition and State.* Study of this aspect of bone is withering but is due for a renaissance. The amount and composition of the bone mineral are affected, among others, by the factor of osteocyte metabolism. There is reason to believe there are variations in mineral composition of the bone in certain diseases, among them being severe and prolonged cardiac failure. There is sufficient evidence to conclude that there is a different physical state of bone mineral present in vivo in addition to the widely known and studied hydroxyapatite. The high degree of instability of this new state, and the fact that it readily converts to hydroxyapatite after death, have prevented its wide recognition in the past.

11) *Osteoid Seams per Cubic Millimeter*· Recently it has been possible to make a true measurement of osteoid seams per unit volume of bone. This came about through the combination of new methods and new physiological understanding of bone. The result is that much finer degrees of qualitative disturbance in osteoid seams are detectable and measureable than previously. We may anticipate that osteomalacia will be found to be the extreme of a continuous spectrum of qualitative disturbances extending from normal bone to Milkman's skeleton.

DISCUSSION

The informed reader has already noted that in the case of many of the preceding factors a variation has not been associated with a known physiological or histological disturbance of bone.

He is correct.

He is correct because these things are too new to have been correlated by previous workers with disease. The presence of the variables has been noted by the writer in one or more pathological states of the skeleton in the course of 7 years' work with fresh, undecalcified bone sections and two years' work with tetracycline labelled human material. The factors are real, but as yet we do not quite know what to do with all of them!

In the future, in stating the quality of osteoporotic bone, or in stating the quality of bone in a testing machine, it will be necessary to evaluate some or all of the variables listed. Other variables not included would require consideration for special purposes.

OSTEOPOROSIS: THE ORIENTATION OF FUTURE BASIC RESEARCH

HAROLD M. FROST, M.D.

INTRODUCTION

In the past 10 years there has been a significant increase in the interest in osteoporoses of various types. This is manifested by a large increase in the number of publications concerning the clinical and fundamental aspects of the disease. Out of this mass of work a pattern is emerging. The pattern reveals the route which future productive work will follow and, in fact, is already following. The orientation of this presentation is histopathophysiological. Significant clinical aspects of interests to many will be omitted.

In order to present the emerging pattern briefly and pungently some basic ground must be reviewed so that the writer and the reader are semantically in tune. This is necessary because the very term, osteoporosis, means different things to different people. We will be concerned from now on with a limited aspect of the problem of genesis of the osteporoses: the relative alterations in bone formation and resorption that are immediately responsible. The more fundamental factors which are in turn responsible for the immediate factors are more capably handled by other investigators.

First the concept of skeletal balance must be presented.

SKELETAL BALANCE

A) Consider all the bone in a single human skeleton. If the volume of the marrow spaces, the vascular channels and the lacunar and canalicular spaces were subtracted, the remainder will be the total absolute bone volume of this skeleton. In a so-called standard man this volume amounts to about 1400 cc. It is preferable that the total amount of bone in any given skeleton be thought of as a volume rather than as a weight because in a given unit volume of bone the weight may vary as the result of variations in the amount of mineral deposited in the bone matrix. As far as is known the amount of matrix in a unit volume of bone does not vary throughout the life history of the unit volume.

The result of these facts is that a given absolute unit volume of bone implies a proportional amount of matrix present in the unit volume, and implies that regardless of how much variation there may be in the degrees of mineralization, the matrix is contant both in volume and in mass.

B) Bone is formed through the actions of cells known as osteoblasts. The osteoblastis form bone matrix. Mineral is subsequently deposited in the matrix, but the prior existence of the matrix is essential for this. Therefore, the direct result of osteoblastic activity is bone matrix. An indirect and necessarily subsequent event is mineralization of that matrix.

If we wish to measure the result of osteoblastic activity, we must measure the volume of bone matrix formed in unit time. This is a true measure of osteoblastic

activity and is not subject to unpredictable variations or qualifications by disease states which affect the degree of mineralization of matrix.

C) Bone is destroyed by cells known as osteoclasts. Just as bone formation is the direct result of osteoblastic activity, and of no other type of biological activity, so bone destruction is the direct result of osteoclastic activity and of no other type of activity.

D) Osteoblastic activity is therefore skeletal anabolic activity and *osteoclastic activity is skeletal catabolic activity*. This may be simply written as the A/C ratio.[2]

E) Skeletal balance is the dynamic state of the total bone volume. As with nitrogen balance, a positive skeletal balance means net accretion of bone matrix and a negative skeletal balance means net loss of bone matrix. Skeletal balance is therefore more than unity, or less than unity. While shifts in the mass of bone mineral of like sign usually accompany shifts in skeletal balance, they do not

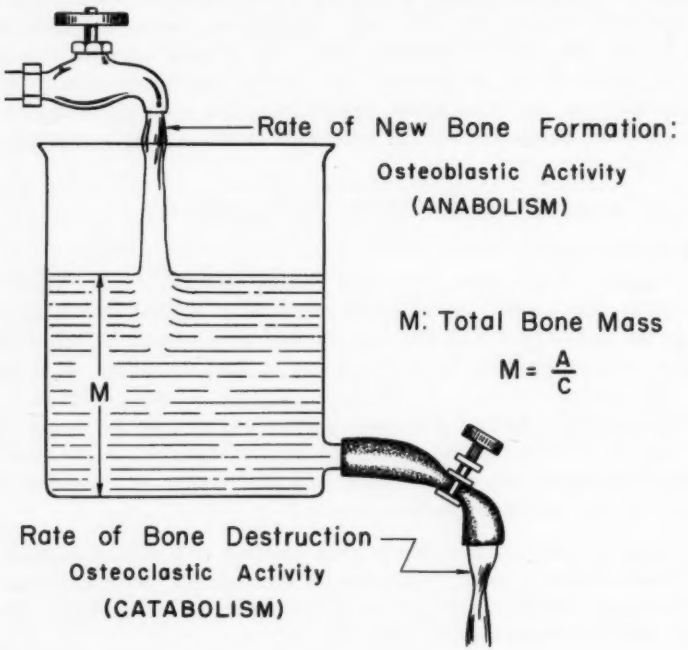


Figure 1

Skeletal balance: A schematic representation of skeletal balance. The amount of fluid in the container represents the amount (absolute bone volume) of bone in the skeleton. When new material is constantly being poured in (anabolism) and old material is constantly flowing out (catabolism), then the total amount of fluid — or bone — is the *result* of the past state of balance between inflow and outflow. Whether the volume of fluid is changing or constant at any one time depends on the balance between inflow and outflow at that time. This is the idea embraced by the term: skeletal balance.

Too little fluid (analagous to osteoporosis) may occur as the result of increased outflow, of decreased or arrested inflow, or of unequal increase in both.

always do so. Therefore methods which measure the total volume of bone matrix will always be correct indicatrixes of skeletal balance. Methods that measure the mineral skeletal mass on the other hand will sometimes be in error. (Fig. 1,2).

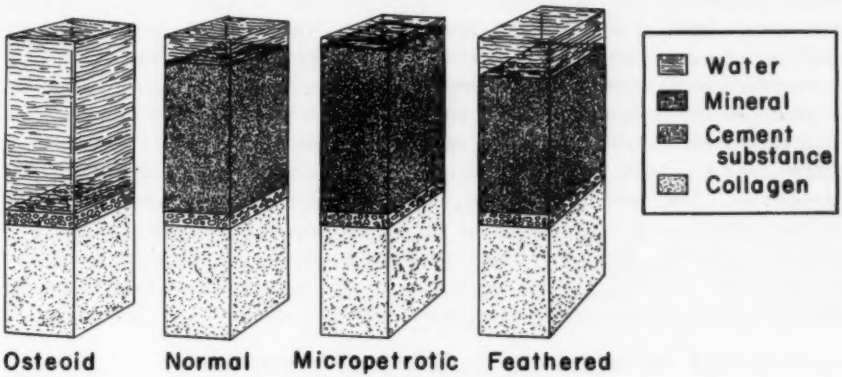


Figure 2

Schematic representation of a *unit volume*, or standard quantity of bone. Note that in all four cases illustrated the organic fraction of bone (collagen and cement substance) is the same in volume. The amounts of mineral and of water vary widely however. Water content is at its largest in newly formed osteoid, while mineral content is at its largest in micropetrotic bone.
Because mineral is appreciably heavier than either water or the organic part of bone, an attempt to illustrate the present situations in terms of *density* lead only to confusion and obscure the true situation.
The *volume* of bone in a skeleton is the correct parameter of measurement for expressing and considering the events involved in the osteoporoses. This volume is dependent on the volume of organic matrix within very narrow limits and *is not affected* by differences in degree of mineralization.

NEGATIVE SKELETAL BALANCE

In the above terms a negative skeletal balance will, given sufficient time, lead to a decreased total absolute bone volume (as well as a decreased total bone mass). The mechanism by which skeletal balance may be negative is of interest, because there is more than one.

A) There may be increased catabolism with normal anabolism.

B) There may be decreased anabolism with normal catabolism.

C) There may be a simultaneous decrease in anabolism and increase in catabolism.

D) There may be unequal increase in both anabolism and catabolism.

E) There may be unequal decreases in both anabolism and catabolism.

OSTEOPOROSIS

As a working definition, let us define osteoporosis in morphological terms: a decreased total absolute bone volume. This of necessity also constitutes a decrease in total bone mass because matrix cannot be absorbed without absorption of the mineral normally found in the matrix.

Osteoporosis as defined above may then occur, *in theory*, by any of the five mechanisms outlined above.

How is osteoporosis supposed to occur *in actuality?* Obviously this depends on the disease in question. In osteogenesis imperfecta there is a congenital decrease in lamellar bone anabolism, while in hyperthyroidism there is an acquired increase in skeletal catabolism. In postmenopausal osteoporosis and in senile osteoporosis there is — supposedly — a decrease in skeletal anabolism. One current theory holds that a major factor accelerating skeletal anabolism is the supply of protein anabolic hormones, exemplified by testosterone and estrogen. Since the endogenous supply of these hormones is known to decrease with advancing age it was postulated that a related decrease in skeletal anabolic activity — meaning formation of bone matrix — necessarily follows, and this leads to the clinical syndromes termed postmenopausal and senile osteoporosis. This same theory holds that the protein catabolic hormones — the cortisone group — do not decrease, or do not decrease in proportion to the protein anabolic hormones. Since the protein catabolic hormones are supposed to be one of the factors affecting skeletal catabolism, osteoporosis would, in theory, be the inevitable result of these combined hormone shifts and effects.

This theory has not been proved. The related facts are these:

A) Estrogens do cause an increase in bone mass in mice and some birds, and originally analogy was made between these animals and man. The original observations were accepted without realizing that the increase in bone mass in these animals was due mostly to an increase in *fibrous* bone, not lamellar bone; while the disease of osteoporosis in man is the result of insufficient lamellar bone in the skeleton.[4] Recently it was learned that there are major differences in the local and homestatic controls of fibrous and lamellar bone formation in man, so that an effect of a governing factor on one type does not necessitate a similar effect on the other type of bone.

B) Finally, and conclusively, the majority of humans given anabolic hormones do not produce more bone in their skeletons even after several years of continuous treatment; some humans treated with these hormones continue to become more severely osteoporotic; rarely does a case produce a demonstrable increase in bone volume and mass. Newer theories based on protein and calcium intake effects are now current and enjoy some clinical success.[5-8]

C) Methods of measuring the quality of osteoporotic bone have had designed into them sufficient insensitivity to prevent recognition of at least two recently recognized bone diseases which are characterized by under- or over-mineralization of matrix. These diseases are feathering and micropetrosis. The methods referred to also had designed into them an inability to recognize a bone composed in part of overly mineralized and in part of undermineralized bone matrix, a combination which has been repeatedly found in aged adult axial skeletons in this laboratory with sensitive methods of evaluating bone quality. A number of other qualitative variables which might exist in osteoporotic skeletons have not been evaluated as yet — too new.

Accordingly the statement that the quality of bone found in human osteoporosis is normal cannot be critically accepted. The quality of osteoporotic bone may not be as grossly abnormal as it is in severe human osteomalicia, but it may be and often is abnormal to lesser degrees and in different respects.

D) It is widely believed that a human osteoporotic patient must be in negative skeletal balance and thus in negative calcium balance. In spite of this belief, accurate balance studies are increasingly revealing that this is not necessarily so. Osteoporotic patients may be in negative calcium balance, or they may be in the balance region of unity, or they may actually be in positive calcium balance.[5,6] Tracer studies have frequently revealed that calcium turnover is increased or normal, and less frequently indicated that calcium balance was negative as postulated. The essential fact overlooked is that skeletal balance as defined here need not always vary with calcium balance since calcium balance is affected by factors in addition to the state of skeletal balance.[5,6]

AGE AND MEASURED OSTEOBLASTIC ACTIVITY

This laboratory is publishing the results of measurement of human osteoblastic activity in a series of cases from birth to over age 70. Osteoblastic activity is at a minimum at age 30 or so and thereafter increases. Osteoblastic activity is increasing during the time in life when most people are gradually losing absolute bone volume.

Since the measurements reveal increasing osteoblastic activity at a time when the endogenous supply of protein anabolic hormone is known to be decreasing, there is only one conclusion: the protein anabolic hormones do not accelerate osteoblastic activity. On the other hand by similar measurements it has been possible to show that the cortisone group of hormones does indeed depress osteoblastic activity, often profoundly.

The measurements quoted, while not in agreement with the protein anabolic theory of the genesis of osteoporosis, are in agreement with the observed clinical behavior of patients' skeletons under the influence of exogenous anabolic and catabolic hormones.

OSTEOPOROSIS: A SIGN, NOT A DISEASE

It is necessary to face the fact that not as much is known about osteoporosis as we thought. In fact, relatively little is known about the genesis, therapy, and nature of the disease when one strips speculation away to find the underlying core of hard facts.

It has been shown that there are at least five basic mechanisms by which a skeleton can go into negative balance.

Available data indicate that at least in so-called senile osteoporosis the cause must be an increase in osteoclastic activity. This increase is out of proportion to the measured increase in osteoblastic activity occurring after age 30.

It is probable that examples of each of the five possible mechanisms of osteoporosis exist in clinical practice. Their recognition will require that the individual interpreting data be aware of these possibilities and aware of the fact that the possibilities are definitely open. Methods need to be devised to investigate the mechanisms actually existing in a given case of osteoporosis.

Osteoporosis is not a disease. Instead it is a sign, the net result of some effect on skeletal balance. This effect is due to an alteration in osteoblastic activity,

osteoclastic activity, or both. These alterations in turn are caused by more subtle and more fundamental physiological alterations and it is the latter alterations and their causes that we must attempt to detect in the future. Otherwise we will not understand what causes the sign known as osteoporosis.

THE FUTURE OF OSTEOPOROSIS

When the fundamental factors governing osteoblastic and osteoclastic activities are found, osteoporosis will assume its proper place in our texts as the *result* of a disease rather than as a disease entity unto itself. Consideration of osteoporosis as a disease entity will die out. With its recognition as a sign will come the realization that the sign is due to a spectrum of etiologies rather than one, or two or such.

To understand the causes of osteoporosis attention must be directed in the future to quantitative osteoblastic and osteoclastic activity. It will be necessary to develop methods of measuring skeletal accretion and resorption, first under laboratory conditions, then under clinical conditions. It is likely that such methods will reveal a spectrum of osteoporoses of unexpected variety and complexity.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases. Philadelphia, Saunders, 1958.

2. Casagrande, P. A., and Frost, H. M.: Fundamentals of Clinical Orthopedics, New York, Grune & Stratton, 1953.

3. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

4. Frost, H. M.: Observations of fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

5. Nordin, Christopher: Personal communication.

6. Nordin, Christopher: Unpublished work.

7. Smith, Richmond: Personal communication.

8. Smith, Richmond: Unpublished work.

(Contents continued from front cover)

IV. Measurement of the Diffusion Pathway Between Osteocyte Lacuna. 137
HAROLD M. FROST, M.D.

6. Human Haversian System Measurements. 145
HAROLD M. FROST, M.D.

7. Physical Characteristics of Bone. Introduction. 148
HAROLD M. FROST, M.D.

I. The Existence of Plastic Flow in Vitro. 149
HERBERT ROTH, B.A., HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA. B.S.

II. Biphasic Elastic Behaviour of Fresh Human Bone. 153
HERBERT ROTH, B.A., HAROLD M. FROST, M.D., AND ANTHONY R. VILLANUEVA. B.S.

III. A Semimicro Measurement of Unit Shear Stress. 157
HAROLD M. FROST, M.D., HERBERT ROTH, B.A., AND ANTHONY R. VILLANUEVA. B.S.

IV. Microscopic Prefailure and Failure Patterns. 163
HAROLD M. FROST, M.D., HERBERT ROTH, B.A., AND ANTHONY R. VILLANUEVA. B.S.

8. Observations on Passive Transfer of Bone Mineral in Man and Nucleation. 171
HAROLD M. FROST, M.D.

9. Evidence for the Existence of Two Physical States of Bone Mineral. 178
HAROLD M. FROST, M.D.

10. Known Qualitative Variables in Human Bone. 180
HAROLD M. FROST, M.D.

11. Osteoporosis: The Orientation of Future Basic Research. 183
HAROLD M. FROST, M.D.